EARLY AND LATE EFFECTS OF DAILY TREATMENT WITH PREGNANT MARE SERUM UPON THE OVARY OF MICE OF THE A STRAIN [1]

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THREE PLATES (TWENTY FIGURES)

Evidence has been presented (Pfeiffer and Hooker, '42) that altered interstitial cells are the source of the androgen produced by the ovaries of mice of the Strong A strain under continuous treatment with large doses of pregnant mare serum. These cells appeared to be derived from old corpora lutea which became subdivided into nests of cells by ingrowths of connective tissue. When first formed these interstitial cells were characterized by eosinophilic granular cytoplasm with no vacuoles, and did not secrete male hormone. Later these cells became vacuolated, simultaneously with the onset of production of androgen by the ovary; and as their vacuolation increased, the level of ovarian androgen rose.

Although the intermediate stages indicated that these interstitial cells originated from the old corpora lutea, the possibility that some, and perhaps many of them arose by direct transformation of stromal cells could not be excluded. This possibility was emphasized by the observation of Cole ('36)

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that in the immature rat the ovarian interstitial cells hypertrophy and increase in number under the influence of pregnant mare serum in the absence of and previous to the first ovulation and luteinization. Accordingly, it was necessary to examine the effects of PMS upon the ovary of the immature mouse, with particular reference to the interstitial cells.

A temporal relation was evident in the earlier experiment between the onset and continuation of production of androgen on the one hand, and the appearance and increase in numbers of vacuolated interstitial cells on the other; but no observations were made as to the character of these cells at the termination of androgen production. In this connection Hill ('41) has reported that ovarian grafts producing androgen under the influence of a cool environment cease to do so at about the age at which senile changes ordinarily occur in the mouse.

The present experiments were undertaken in an attempt to clarify these two points as well as to study the ovary after very long-continued treatment with PMS.

MATERIALS AND METHODS

Female mice of the A strain (Strong, '36) were used in all of the experiments. One group of twelve animals received daily injections of 12.5 R.U. of pregnant mare serum (PMS) for the first 10 days after birth. The daily dose was then increased to 25 R.U. A second group of forty-four mice did not receive treatment until they were either 21 or 35 to 40 days old (table 1), at which time daily injections of 25 R.U. were started. Twenty-one of the second group were treated uninterruptedly for 3 to 15 months. When the injections were started at birth the animals were segregated from their mothers when about 28 days of age. In injecting the very young animals, the needle was inserted for a considerable distance under the skin to prevent leakage of the hormone when the needle was withdrawn. The hormone was administered in such concentration that 25 R.U. were contained in 0.1 cc. The animals were maintained on a diet of Purina fox

chow pellets and fresh water. Most of the tissues were preserved in Bouin's fluid, sectioned at 8 μ and stained with Erhlich's hematoxylin and triosin. The ovaries of a few of the oldest animals were fixed in 10% neutral formalin and stained with either Bennholt's Congo Red or Mayer's stain to determine whether any of the degeneration in the ovaries involved deposition of amyloid.

OBSERVATIONS

There was no evidence that the extended treatment with PMS to which the mice were subjected had any direct toxic effects. However, a number of changes were encountered which have been produced in mice by long-continued administration of large amounts of estrogen (Gardner, '40) and by an abnormal release of endogenous estrogen (Pfeiffer, '39). Urinary retention and distention of the bladder, often accompanied by hydronephrosis, occurred in all of the animals treated for more than 150 days. Approximately one-half of the animals developed pyometra after 150 days. Mammary tumors appeared in six of the seven animals surviving more than 8 months' treatment, and one cervical tumor was observed after 12 months' treatment. Endosteal hyperossification of the skeleton, as indicated by the macroscopic structure of the femur, was present in most cases; and the pubic symphysis was usually replaced by a ligament which attained a length of 2 to 5 mm. under the longer periods of treatment. These several changes were doubtless the result of steady production of estrogen under the influence of the injected PMS. Stunting of growth was evident in the animals treated from birth, and not when treatment was started at later ages.

Effects of PMS during the first 30 days of life. In the animals receiving PMS from birth there was no evidence of ovarian stimulation during the first 12 days (cf. figs. 1 and 3 with figs. 2 and 4). From the fifteenth until the thirty-fifth day, only follicular growth with concomitant production of estrogen, but without ovulation, occurred. The vagina opened on the fifteenth or sixteenth day and its epithelium became

cornified and thickened during this period. Significant ovarian enlargement was evident by the twentieth day (fig. 5). Thereafter its hypertrophy was progressive (figs. 6 and 7), and by the thirtieth day the ovaries were about five times as large as in untreated animals of the same age.

The ovarian hypertrophy during this period consisted entirely of follicular growth, the onset of which was suggested as early as the tenth day of treatment by thickening of the stratum granulosum and by intercellular accumulations of fluid in the granulosa. Small antra were present on the fifteenth day, and large follicles were present in considerable numbers on the twentieth day. Increasing numbers of large follicles were found through the thirtieth day. Beginning on the twentieth day the follicles showed certain unusual features. The granulosa was often very thin opposite the cumulus. Many large follicles lay in the deeper portions of the ovary, and their appearance suggested that they were becoming cystic. After the twenty-fifth day a few of the follicles were hemorrhagic.

Beginning on the twentieth day hypertrophy of the cells of the theca interna was apparent, and a few of these cells contained mitotic figures. At 30 days some of the thecal cells had enlarged sufficiently to resemble luteal cells (fig. 8), possibly constituting the first evidence of luteinization. During this entire period the ovarian stroma remained essentially unchanged in relative amount and in character. The stromal cells showed no hypertrophy; and interstitial cells, i.e., epithelioid cells in the stroma, were entirely absent.

A cornified vaginal smear was consistently obtained from the time of establishment of a patent vagina, and the vaginal epithelium was thick and cornified. By the fifteenth day the uterine and tubal epithelia had become slightly heightened. By the twentieth day the uterus had become distended with fluid and edematous, and the epithelium was tall notwithstanding the distention. By the thirtieth day the uterus had reached mature size, had well developed glands and showed a fairly marked irritability. These uterine and vaginal changes can be

attributed to the action of estrogen. In keeping with the absence of corpora lutea, the stromal nuclei of the endometrium were not vesicular as they are under the influence of progesterone (Hooker, '40).

Effects of PMS when treatment was started at 21 days of age. The animals which did not receive injections of PMS until they were 21 days old showed the same genital morphology on the twenty-fifth and thirtieth days of life as the animals whose treatment began at birth, although in the latter animals the changes began before the twenty-first day.

Early effects of PMS when treatment was started at 35 to 40 days of age. Appreciable luteinization, and hence accumulation of interstitial cells, did not occur before the thirty-fifth day of life, even in the animals treated from birth. Consequently, treatment of most of the animals was started at the thirty-fifth to the fortieth day. The sequence of events from this time through 75 days of treatment has already been described (Pfeiffer and Hooker, '42), and in the present investigation only enough animals to confirm the earlier observations were sacrificed during this period.

For purposes of orientation, the changes taking place during this period will be reviewed briefly (table 1). The first response of the ovary was a generalized exaggeration of all of the normal events in the ovary. There was an increase in follicular growth with resultant supernumerary ovulation and formation of corpora lutea. Associated with the excessive number of follicles, estrogen was the predominant secretion of the ovary during the first 21 days of treatment. All of the corpora lutea persisted intact longer than the normal cyclic corpus, and by the twenty-first day of treatment they became so numerous that they were the principal component of the ovary. Correspondingly, progestin now became the predominant ovarian hormone. This condition persisted until sometime between the thirtieth and thirty-fifth days of treatment.

Early in the period of treatment eosinophilic granules appeared in the cytoplasm of the cells of the older corpora lutea (fig. 10), and ingrowth of connective tissue from the periphery

TABLE 1

Ovarian response in mice of the A strain to chronic daily treatment with 25 R.U. of pregnant mare serum.[1]

NUMBER OF ANIMALS	AGE AT WHICH TREATMENT WAS BEGUN	DAYS OF TREATMENT	PRINCIPAL RESPONSE	PREDOMINANT CELL TYPE	PREDOMINANT HORMONE SECRETED
	(days)				
4	0	0–15			
6	0	20–30	follicular development	granulosa	estrogen
2	0	30–35	follicles beginning luteinization	granulosa; some luteal cells	estrogen
5	21	0–15	follicles beginning luteinization	granulosa; some luteal cells	estrogen
8	35–40	0–21	supernumerary ovulation; corpus luteum formation	granulosa and luteal cells	estrogen
5	35–40	21–35	excessive luteinization	normal luteal cells and granular eosinophilic cells	progestin
5	35–40	36–90	accumulation of large interstitial cells	vacuolated eosinophilic cells	androgen
6	35–40	90–150	accumulation of large interstitial cells	granular eosinophilic cells	estrogen and some progestin [2]
8	35–40	155–250	accumulation of large interstitial cells	heavily granular eosinophilic cells	estrogen and some progestin
7	35–40	260–455	atrophy	heavily granular eosinophilic cells	little hormone released

[1] When animals were started at birth they received 12.5 R.U. of PMS daily for the first 10 days and 25 R.U. daily thereafter.

[2] Androgen was also usually produced during some of this period.

of the corpora lutea divided the corpora into variously-sized nests of cells (fig. 9). As early as the tenth day of treatment small vacuoles were present in a few of the cells with eosinophilic granules, and slight fibrosis of the uterine tunica propria suggested secretion of slight amounts of androgen. Androgen did not become the predominant hormone secreted by the ovary until the thirty-fifth day of treatment, at which time eosinophilic granular interstitial cells became the principal cellular element of the ovary. Vacuolation of these cells was now marked. The ovaries continued to produce androgen until at least the ninetieth day of treatment, and probably much longer in some cases. Although new eosinophilic granular cells were constantly being added, the vacuolated eosinophilic cells showed no evident increase in number after the thirty-fifth day of treatment. Their vacuoles, however, became more numerous and much larger (figs. 11 and 12). It is not impossible that extreme vacuolation indicated approaching cellular exhaustion, since highly vacuolated cells were present in greatest numbers toward the end of the period of androgen production and were no longer present when androgen production had ceased.

Late effects of PMS when treatment was started around 35 days of age. Secretion of androgen ceased sometime between the ninetieth and one hundred and fiftieth days of treatment. It is impossible to designate the time more closely because of the small number of animals autopsied during this period (table 1), and unquestionably there were individual variations in this respect. All of the animals still alive after 150 days of treatment were given subcutaneous transplants of seminal vesicles, and in none of the grafts was there any stimulation to indicate action of androgen. Indeed, the grafts could seldom be found at exploratory operations, whereas in the presence of male hormone, grafted seminal vesicles persist and grow in the rat (Price, '41). Follicles continued to develop and normal corpora lutea still formed during the period between the nintieth and one hundred and fiftieth days of treatment; and, correspondingly, estrogen was now the predom-

inant secretion of the ovary, although some progestin was also secreted.

The ovaries steadily increased in size through 150 days of treatment, attaining a size four or five times that of normal ovaries. The major contribution to the hypertrophy was interstitial cells arising from corpora lutea. Some of the enlargement, however, was due to edema and increasing vascular congestion dating from the early days of treatment.

After 150 days of treatment the eosinophilic granular cells continued to form as before, but they no longer became vacuolated; instead they became more densely granular. These cells were larger than the typical eosinophilic granular cells which were present earlier (fig. 10), and their granules were larger and more strongly eosinophilic. A few of these very granular cells (fig. 13) were found as early as the sixtieth day. After the cessation of androgen production they became numerous and were the predominant cell type (fig. 14) in the ovary between the one hundred and fifty-fifth and two hundred and fiftieth days of treatment, although the time at which they reached ascendancy apparently varied considerably among individual animals. There was no evidence that they secreted any hormone. This condition persisted until treatment had continued for about 1 year, when the ovary began to evince an increasing incapacity of developing many follicles. Thereafter, the ovary was composed almost entirely of densely granular cells, some of which had begun to undergo a hyaline type of degeneration (fig. 18). Often preceding this degeneration many large brown cells (fig. 15) made their appearance, especially if there had been marked vascular congestion. It is possible that these cells were macrophages and that the brown particles giving them their characteristic color were hemosiderin. The degeneration of the granular cells progressed fairly rapidly, with the result that large islands of hyalinized tissue were soon present (fig. 16). These islands became larger and coalesced, enclosing gradually receding areas of heavily granular cells. The more centrally located cells in these patches of granular cells appeared viable, while the nuclei of

most of the outer cells were pycnotic and the cytoplasmic granules less distinct (fig. 17). Hyalinization increased to the extent that the ovary, which may have attained a size six or eight times that of the normal ovary (cf. figs. 19 and 20), was composed almost entirely of this material. Only the presence of an occasional group of a dozen or more granular cells with pycnotic nuclei and an infrequent, degenerating young follicle served to identify this mass as an ovary. It is doubtful that any hormone was secreted by the ovary after hyaline degeneration set in. Tests for amyloid in these hyalinized ovaries were negative.

DISCUSSION

It is evident, as shown by Hill ('41), that after a suitable interval in an appropriate environment the ovary of the mouse secretes male hormone at a relatively high level for some time and then ceases to do so. The administration of PMS both hastens the onset of male hormone secretion and shortens the period of its production. Under the conditions of Hill's experiments ovarian grafts stopped producing androgen upon reaching a chronological age equivalent to that at which reproductive activity normally ends in the female mouse, suggesting that the termination of androgen production was associated with senility. Under the influence of PMS, on the other hand, the ovary completed the period of production of androgen at an age at which reproductive activity is still high in the normal female.

The disappearance of the vacuolated eosinophilic cells coincidentally with the discontinuation of production of androgen and the persistence in abundance of the unvacuolated, densely granular cells suggests that while the factor postulated (Pfeiffer and Hooker, '42) as controlling the formation of granular interstitial cells had remained active, the factor postulated as evoking secretion and vacuolation had become inoperative. Following this reasoning, the duration of production of ovarian androgen may be an expression of the effective period of the latter of these factors. Thus, the essen-

tial effect of the PMS may be an earlier release of the postulated secretory factor and an earlier termination of its effectiveness than under the conditions of Hill's experiments. The earlier termination may perhaps be due to release of this factor at a higher level during the active period.

It is possible that this factor is produced by the hypophysis, and that it is released in response to some stimulus other than the injected PMS per se. This possibility is in keeping with the secretion of androgen by ovarian grafts in males not under the influence of exogenous gonadotropins (Hill, '37). Under this assumption, which must be tested with hypophysectomized animals, the termination of effectiveness of the factor might be due either to exhaustion of the hypophysis in respect to this function or to a specific exhaustion of the ovary. The possibility that the androgen-producing cells become exhausted is consistent with the history of the ovary during later periods of treatment, a history which suggests that the disappearance of the vacuolated interstitial cells is the first step in the gradual exhaustion of the entire ovary, inasmuch as failure of vacuolation precedes any evidence of general unresponsiveness of the ovary. Ultimately there was a gradual reduction in the number of primary follicles, indicating a further and progressive ovarian exhaustion. The secretion of small amounts of estrogen and progestin subsequent to the cessation of male hormone production corresponds with the continuation of both follicular development and luteinization until shortly before hyaline degeneration set in.

The failure of the ovaries to respond to the gonadotropic principle before the twelfth or fifteenth day is in agreement with the early work on this subject (Smith and Engle, '27; Hill, '32; Wiesner, '32; Pfeiffer, '34) indicating that a certain degree of ovarian maturity is prerequisite to response to gonadotropins. Although the follicular response without ovulation or luteinization in the immature animals corresponds with observations on the immature rat treated with PMS (Cole, '36), the early interstitial cell response reported in the rat seems to be quite lacking in the mouse. The failure to

produce hypertrophy and increase in the number of stromal cells in the immature mouse indicates that here luteinization is essential for the formation of interstitial cells. It follows, therefore, that most, if not all, of the granular or vacuolated ovarian interstitial cells in the mouse arise by subdivision of corpora lutea rather than by metamorphosis of stromal cells, supporting the conclusion arrived at on study of the intermediate stages in the formation of these cells (Pfeiffer and Hooker, '42).

The stunting which resulted when administration of PMS was started at birth was probably not a direct effect of the PMS, but instead could be attributed to the handling of the animal, to the injection of amounts of solution so large in comparison to the size of the animal, or possibly to inhibition of growth caused by the estrogen evoked by the PMS (Deanesly, '39).

SUMMARY

1. In female mice of the A strain receiving daily injections of 25 R.U. of PMS for as long as 15 months, no toxic effects were observed other than the side effects of continuous action of endogenous estrogen. These effects usually appeared after 150 days of treatment. No refractoriness to PMS was evident at any time.

2. The stimulation of the very immature ovary was delayed in its onset and consisted only of follicular development. After sufficient maturity (30 days or more) the first effect of PMS was a general stimulation of the ovary, with supernumerary ovulation and concomitant evidence of increased estrogen production. There was no evidence of stimulation of the interstitial cells previous to the formation of corpora lutea. The corpora lutea persisted intact for an unusually long period, a condition which, coupled with the rapid formation of new corpora, resulted in a great increase in the amount of luteal tissue and consequently in the release of large amounts of progestin. Progestin production reached a maximum between 21 and 35 days of treatment.

3. The old corpora lutea were subdivided into nests of cells by ingrowths of connective tissue. These cells which normally become stromal cells persisted as eosinophilic granular cells and became vacuolated coincidently with the onset of the secretion of androgen by the ovary. Androgen production was appreciable by the thirty-fifth day and continued beyond the ninetieth day. By the one hundred and fiftieth day both vacuolation of the cells and production of male hormone had ceased.

4. Non-vacuolated cells continued to form after vacuolated cells could no longer be found. The ovaries had by this time reached a size six or eight times that of the normal ovary. Some follicular development and luteinization still continued and small amounts of estrogen and progestin were produced. After 200 days of treatment the ovaries were composed primarily of the heavily granular, unvacuolated, eosinophilic cells.

5. Late in the period of treatment progressive ovarian exhaustion set in, the first stage apparently being an inability, after 150 days, to maintain vacuolation of the interstitial cells. After 12 months of treatment ovarian exhaustion was clearly shown by reduction in the number of primary follicles. Ultimately, the ovaries, which now consisted almost entirely of granular interstitial cells, underwent hyaline degeneration. After significant reduction in the number of primary follicles, no evidence of the production of any sex hormone was obtained.

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EXPLANATION OF PLATES

All figures are photomicrographs of ovaries of mice of the A strain, sectioned at 8 μ, and stained with Ehrlich's hematoxylin and triosin

PLATE 1

EXPLANATION OF FIGURES

1 From an untreated mouse 10 days of age. × 75.

2 From a mouse of the same age which had received 12.5 R.U. of PMS daily from birth. The apparently larger size as compared with the control (fig. 1) is due entirely to the plane of section. The interstitial cells and the state of development of the follicles are the same in both cases. × 75.

3 From an untreated mouse 15 days of age. × 75.

4 From a mouse of the same age which had received 12.5 R.U. of PMS daily for the first 10 days and 25 R.U. daily for the remaining 5 days. No effect of the treatment is evident. × 75.

5 A portion of the ovary of a 20-day-old mouse treated with 12.5 R.U. of PMS daily for the first 10 days and with 25 R.U. daily thereafter. Follicular growth with no luteinization or interstitial cell development is shown. × 75.

6 From an animal 25 days of age after the same treatment. The response is still limited to follicular growth. The thinning of the granulosa opposite the cumulus suggests that these follicles are becoming cystic. × 75.

7 After 30 days of the same treatment. In some follicles the granulosa is of normal thickness, while in others it is quite thin. The latter follicles are becoming cystic. Nor corpora lutea were present. × 75.

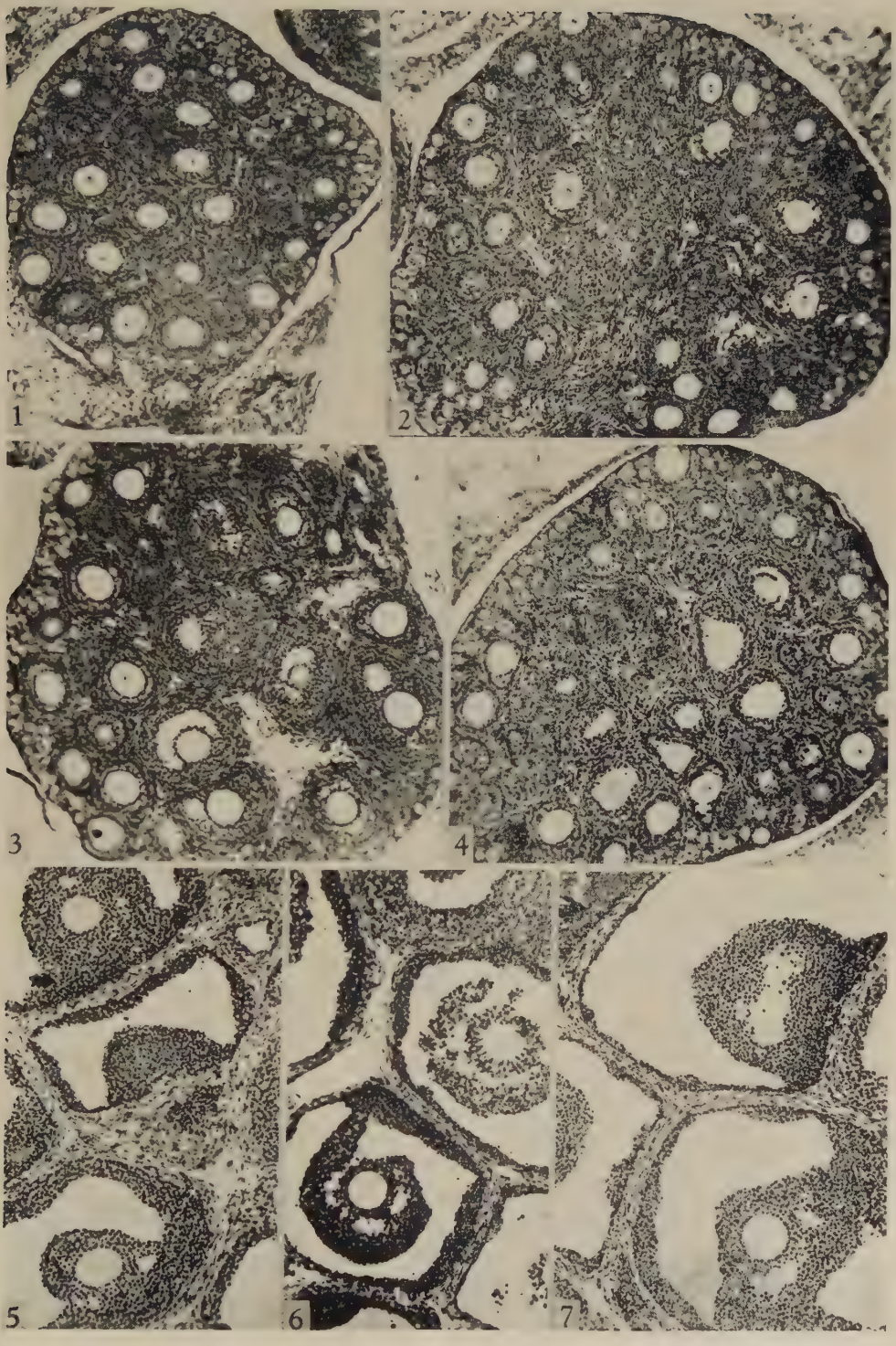
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PLATE 2

EXPLANATION OF FIGURES

8 A portion of a follicle in the ovary in figure 7 showing beginning thecal luteinization, or "thecal gland formation." This is the earliest evidence of luteinization. Theca externa at extreme left; granulosa at right; thecal gland between. × 300.

9 A small portion of an old corpus luteum showing the ingrowths which subdivide the corpus into variously-sized masses of cells. This animal was given twenty-one daily injections of 25 R.U. of PMS beginning on the thirty-fifth day of life. × 300.

10 An area of the ovary of an animal which had received 25 R.U. of PMS for 56 days beginning at 35 days of age. A young, relatively basophilic corpus luteum above, and an older corpus with eosinophilic cells below. × 300.

11 A mass of highly vacuolated, eosinophilic cells derived from an old corpus luteum in the ovary shown in figure 10. This ovary was producing androgen. A bit of germinal epithelium is shown at the upper left. × 300.

12 Highly vacuolated, eosinophilic cells in a mass of interstitial cells in the ovary after 70 days' injections of 25 R.U. of PMS from the thirty-fifth day. × 700.

13 Eosinophilic interstitial cells in the ovary of a mouse receiving the same treatment for 60 days. The few, scattered granular cells with large, ellipsoidal nuclei are probably an early phase of the densely granular cells present after vacuolation ceases. × 300.

14 Densely granular interstitial cells which predominate between the one hundred and fifty-fifth and two hundred and fiftieth days of treatment. This animal had received 25 R.U. of PMS daily for 300 days. × 700.

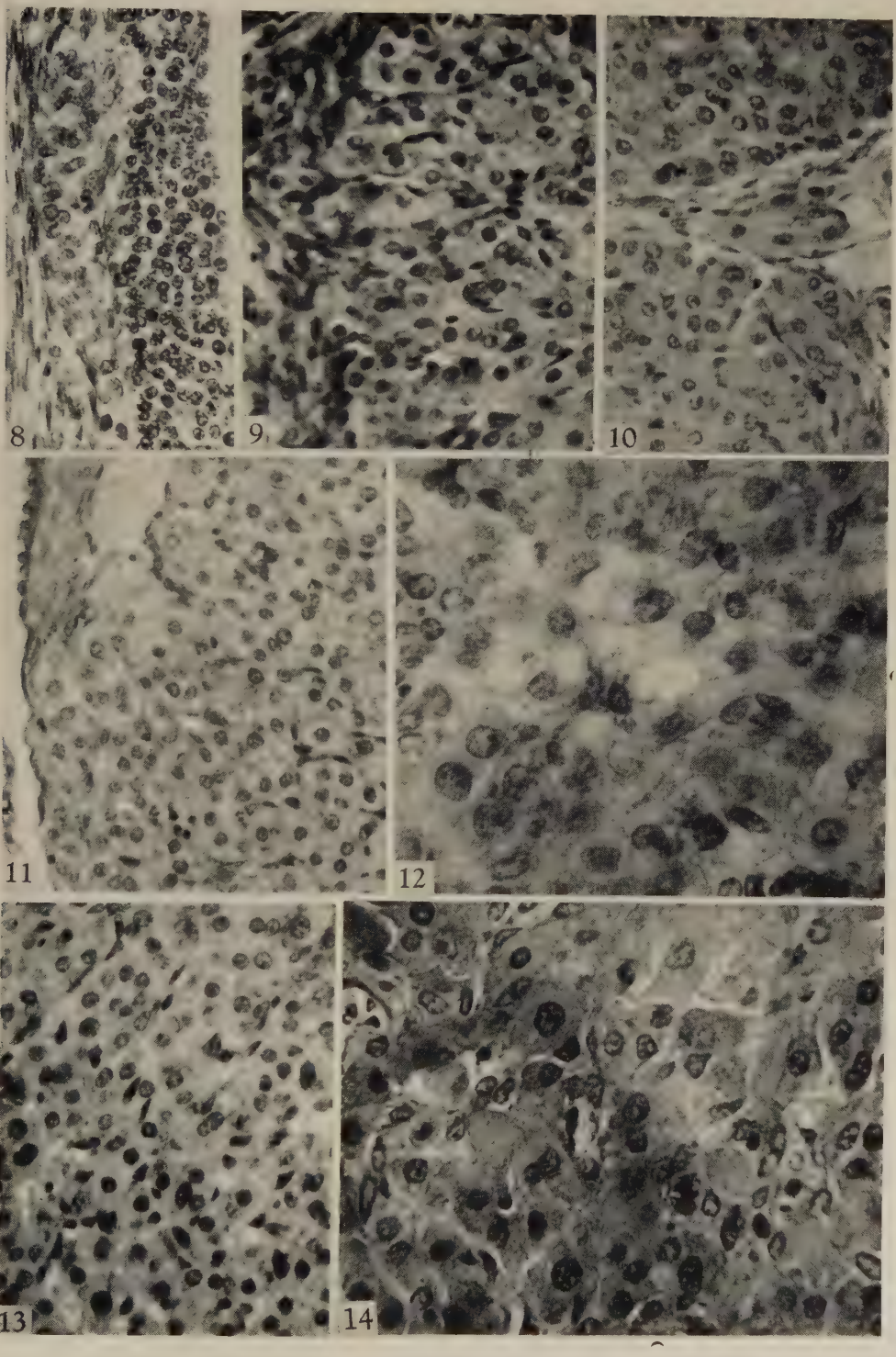
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PLATE 3

EXPLANATION OF FIGURES

15 A small area in the ovary of a mouse treated daily for 400 days with 25 R.U. of PMS. This area is composed almost entirely of large multi-nucleated brown cells. One "cell" occupies the lower central portion of the photograph. × 300.

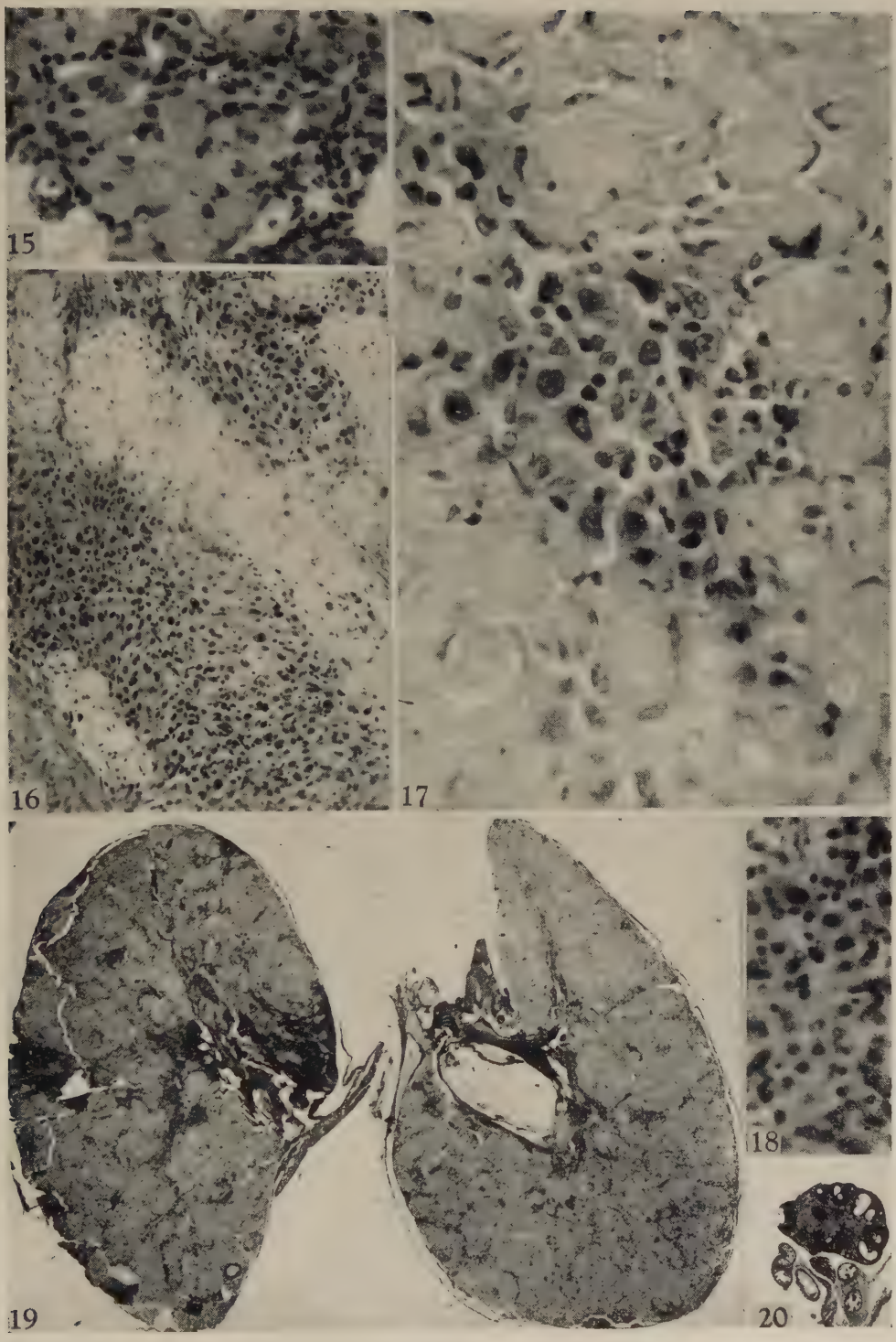
16 A portion of an ovary after 450 days' treatment. Note the areas of degeneration and the frequently pycnotic nuclei of the densely granular cells. × 75.

17 An area of the ovary in figure 16 showing a small island of densely granular cells in an area of degeneration. The central cells are surrounded by cells undergoing degeneration. × 700.

18 An area in the ovary of a mouse which had received injections of PMS for 300 days. Early necrosis is shown which occurs before brown cells and hyalinization make their appearance. × 300.

19 Cross section of both ovaries after 450 days of treatment. These ovaries are composed primarily of hyaline material as in figures 16 and 17. × 10.

20 For comparison, a cross section of the ovary and tube of an untreated, adult mouse of the A strain. × 10.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A METHOD AND APPARATUS FOR OPENING AND CLOSING EGGS WHICH WILL PERMIT TURNING AT REGULAR INTERVALS DURING SUBSEQUENT INCUBATION

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TWO FIGURES

Different workers have used various methods in opening and closing eggs used for experimental purposes. Some papers of interest in this connection are those of Burnet ('36), Clark ('20), Fugo ('40), Fugo and Witschi ('38), Hamburger ('34), Kuo ('32), Martindale ('41), Orr and Windle ('34), Paff ('39) and Rawles ('36).

Any one of the methods suggested was found inadequate in one or more respects for the particular problems of experimental embryology in which the present author was interested. An effort was made to devise a method which would re-establish as near a normal condition as possible for the egg after opening and closing.

All eggs used in experimentation are supported in a horizontal position by means of the knobbed type of paper egg trays used in egg cases of 30-dozen capacity. A small pencil mark indicates the uppermost point of the egg during preoperative incubation, and the eggs are not turned during this period. This static position leads to a slight stratification of the egg contents and guarantees that a higher percentage of the young embryos will be uppermost and in the center of the visual field when the egg is opened at the point marked. Such embryos are easy to approach at any point, whereas embryos resting toward the side of the yolk cause much difficulty and must frequently be discarded.

After the embryos have reached the desired stage of development, about six eggs are removed from the incubator at one time, and immediately turned on the tray into such a position that the mark is now on the under side of the egg and at the lowest point. The yolk mass will then be forced upward by the heavier albumen. An egg is then taken in hand without altering its orientation and after wiping it off with 70% alcohol, a hole is punctured from below at the pencil mark. This prevents any injury to the embryo or rupture of the vitelline membrane with the escape of yolk. This puncture may be made by the use of a sterilized dissecting needle whose needle end has been cut off to a length of 3 mm. The egg is now immediately turned in the hand so that this opening is uppermost. Following this another puncture is made on the blunt end of the egg passing through the shell and outer shell membrane only. On withdrawing the stub needle, the air in the air cell escapes and an equal amount of air passes into the uppermost opening as the yolk mass and albumen settle down and away from the shell. When air rushes into the opening above the yolk, a tough bubble sometimes forms in the albumen. This bubble may be ruptured with a hot dissecting needle after the opening has been enlarged.

The uppermost opening is increased by the use of an ordinary pointed forceps previously sterilized, until it is fairly circular and about 20 mm. or less in diameter. There is no danger of injuring the embryo or yolk mass since there is a considerable air space between the shell at this point and the egg contents. This air cavity is of great advantage in working on the embryo, since it allows the entrance of instruments, vital dyes or saline into the egg without having to withdraw albumen equal in amount to the fluids added. Furthermore, it permits the egg to be tilted through a wide range of angles to bring the embryo into an optimum position without any of the albumen running out.

Following the operation on the embryo a sterilized no. 2 coverglass of 20 mm. diameter is placed over the opening in the egg-shell, and melted ceresine wax is applied between

the coverglass and the egg-shell by means of a camel's hair or mucilage brush. The cerescine is applied all around except at one point where a small opening of about 3 mm. width is left. The air-cell cup is then applied to the blunt end of the egg as shown in figure 1 and with sufficient pressure to force air back into the air cell and against the inner shell membrane. This raises the egg contents up against the coverglass, driving out all the air through the small opening left. By holding the egg and air-cell cup steadily, one can easily paint shut the small opening which remains. When the air-cell cup is removed, the opening to the air cell is also painted shut. Following this, the egg is quickly turned in the hand so that the coverglass is on the bottom surface of the egg. This permits the yolk mass to float directly upward without scraping on the rough edges of the egg-shell where it was opened, and prevents any possibility of the yolk mass being ruptured. More melted cerescine is applied to the egg-shell and coverglass and over its edges to prevent the weight of the egg contents from pushing the coverglass off. Eggs so treated, have been brought back very nearly to the pre-opened condition. There exists a maximum of moist surface for respiratory exchange.

Cerescine wax has a melting point of 60–62° C. which is high enough to prevent any undue softening in the incubator. It is also odorless and pure white, being in all necessary respects an ideal medium for sealing eggs. Ordinary paraffin has been found unsatisfactory because it softens in the incubator and allows the coverglass to drop off, although its whiteness and lack of odor are desirable qualities. Beeswax stands up about as well as cerescine at the incubation temperature but is undesirable because of its increasingly darkening color and odor while being kept hot. Gerlach's mixture (Gerlach, 1884), consisting of two parts of beeswax and three parts of lump resin was tried but found to be inferior to cerescine.

The air-cell cup used above is shown in greater detail in figure 2. It consists of the funnel end of a thistle tube, the thistle portion of which has been cut off and the remaining

stub sealed in a flame. Over the mouth of the funnel is stretched a sheet of dentist rubber dam in the center of which a circular hole has been previously cut by the use of a cork bore or burned with a hot glass rod. The sheet is moored

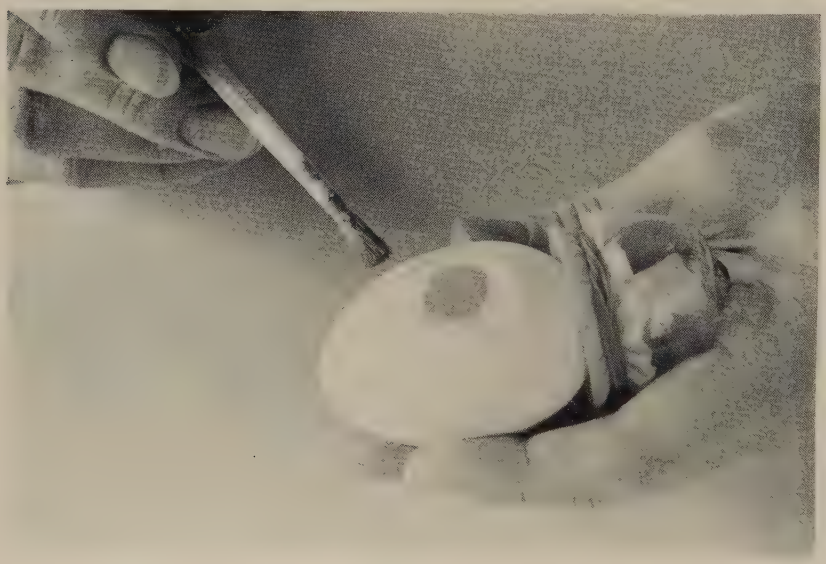

Figure 1

Fig. 2 Construction of egg-cup. 1. Funnel air chamber. 2. Rubber band. 3. Dentist dam. 4. Hole in dam.

below the lip of the funnel with rubber bands. When the air-cell cup is pressed against the egg, the rubber dam makes an air tight seal and the compressed air is driven into the egg thus re-establishing the air cell in its original form.

The egg is placed back into the tray with the coverglass below, returned to the incubator and allowed to develop for the next 2 days without any turning so that the weakness in the yolk mass produced through the rupture of the vitelline membrane at the point of operation on the embryo may be compensated and re-enforced by the extension of the extra-embryonic membranes. Thereafter the egg may be turned to most any position at regular intervals during later days of incubation. During the earlier days of incubation, the embryo will always slip into full view against the cover-glass when the egg is turned with the glass uppermost. This permits a critical examination of the embryo under the binocular during later development, and makes it very easy to discover any dead embryos.

A more substantial cover than the one mentioned above, for application to the egg opening may be very simply made from microscopic slides by placing one slide on another, using the width of the upper one as a measure for length and as a square, and making cuts with the glass cutter. This will make three equal squares from one slide. Glass covers may be recovered by washing successively in water, dioxan, xylol, dioxan and ammonia water to remove the albumen and cerescine.

A number of advantages are combined in this method and may be summarized as follows: (1) After opening and closing the egg, it may be turned regularly during subsequent incubation. (2) The embryo may be observed without any fogging of the coverglass. (3) The embryo is perfectly sealed from outside sources of contamination. (4) A maximum of moist respiratory surface is restored. (5) There is no drying of the extraembryonic membranes. (6) Any abnormal increase in water loss is prevented. (7) None of the egg contents need be withdrawn. (8) Space is provided for operating and

adding materials. (9) The embryo may be approached at its normal site with no injury. (10) No addition of saline is necessary to bring the embryo back to the original level. (11) The air cell is re-established.

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AN EMBRYOLOGICAL MICROMANIPULATOR WITH AN ADJUSTABLE EGG-HOLDER ATTACHMENT

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TWO FIGURES

A number of different types of micromanipulators have been developed for producing very small and accurately controlled movements in experimental work. Most of these instruments used in biological work have found excellent applications in certain phases of cytology and experimental embryology. Among the micromanipulative instruments already in existence may be mentioned those of Barber ('14), Chambers ('22, '23), Peterfi ('23), Taylor ('25), Hansen ('39), Fitz ('31), and Brown ('40). Other similar instruments of interest are those of J. H. Emerson (Cambridge, Mass.) and of some nineteen others whose names and references are cited in McClung ('37).

These instruments are very excellent for certain types of work, but are not satisfactory for the particular problems of experimental embryology in which the author was interested. One of the above-mentioned instruments has the disadvantage of giving a movement of translation in the horizontal plane by a series of zig-zags, produced by turning two screws alternately. Most of the manipulators have the disadvantage of being capable of movements through short distances only and at a slow rate. One instrument allows a radius of movement of only 2 mm.

In view of these facts the author undertook the development of a micromanipulator which would produce a rapid or slow movement (in a straight line) and one of large or

small extent (up to 2 inches in each of the mutually vertical planes), while at the same time retaining accuracy of control and smooth movement.

Other desirable features of this device are as follows. The micromanipulator may be quickly attached or removed from the stage of the dissecting binocular, since only the mirror together with the glass stage need be removed from the scope. The stage of the micromanipulator slips into the same grooves that hold the glass stage and is anchored in the same way. Further, the micromanipulator may be used as a right-handed or a left-handed instrument, depending on the way in which it is slipped onto the scope stage. The instrument may be made into a bilateral manipulator like other instruments, by the simple addition of another unit to the other side. Perhaps very helpful also is the fact that this instrument can be built up quite readily by any handy mechanic making use of parts already in existence, and therefore necessitating little additional expense in making the rest of the needed parts.

The micromanipulator illustrated in figure 1 and shown in position on the binocular ready for use in figure 2, is constructed as here described. The rack and pinion portion of two small dissectors are moored together at their bases with a heavy brass brace. The inner knurl of each is cut off and an extension added to the outer knurl of the left rack and pinion unit, to allow for adjustment from beyond the binocular stage. A brass plate of the same dimensions as the glass stage of the dissector is prepared and a hole placed in its center, through which the upper end of the left rack and pinion unit is passed and moored as was the stage which has been removed from the small dissector. To the top of the right rack and pinion unit is mounted a mechanical stage from which all surplus parts have been removed. On the end of the slide bar of this stage a holder is attached. A holder can be fashioned such as will accommodate many instruments like micropipettes, microelectrodes and microneedles. A further rigidity in the instrument is secured through binding the brass stage to the upper end of the right rack and pinion unit. This

can be done by the use of heavy brass plates. To the top of the left rack and pinion unit any type of operative stage may be added, such as the adjustable egg-holder also shown in figures 1 and 2.

By the use of the knurl on the right rack and pinion unit, the mechanical stage is raised or lowered; the two knurls on the mechanical stage mounted on top provide the two horizontal movements at mutually right angles. The knurl on the

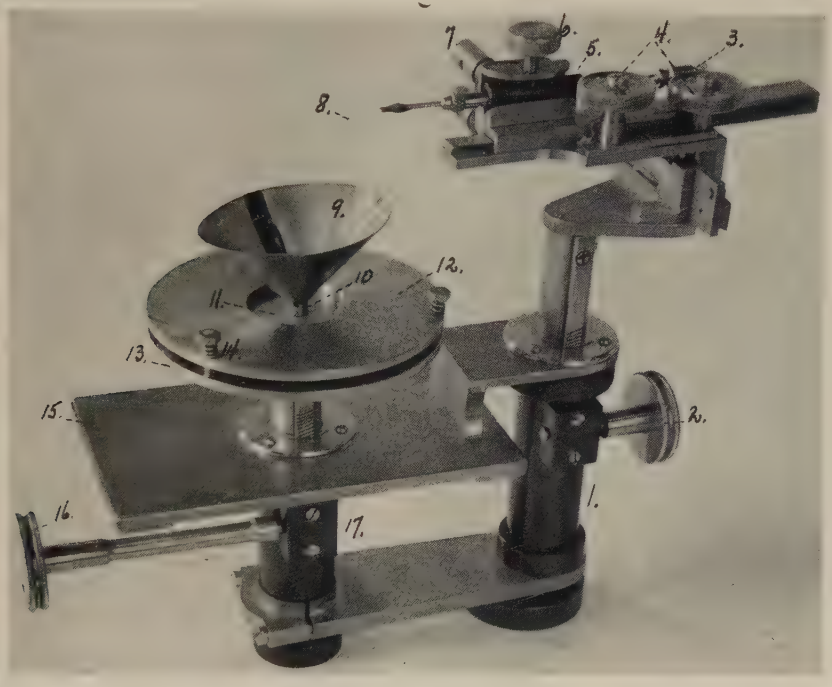

Fig. 1 Micromanipulator with adjustable egg-holder attachment.

1. Right rack and pinion unit.
2. Right vertical adjustment.
3. Binding post on electrode holder.
4. Knurls for adjustment in horizontal plane and at right angles.
5. Rubber insulating sleeve on electrode holder.
6. Electrode holder screw.
7. Insulated binding post.
8. Electrode.
9. Egg-cup.
10. Egg-cup pedestal.
11. Middle disc exposed through hole in upper plate.
12. Upper disc.
13. Lower disc.
14. Pressure spring.
15. Stage of micromanipulator.
16. Left vertical adjustment.
17. Left rack and pinion unit.

left rack and pinion unit provides a means for elevating or lowering the operative stage in conjunction with both the scope adjustments and the manipulated instrument. The various parts of the micromanipulator may be identified by reference to the key given below figure 1.

The adjustable egg-holder attachment about to be described was developed in order to provide a number of essentials as follows: (1) to hold the egg firmly in any given position

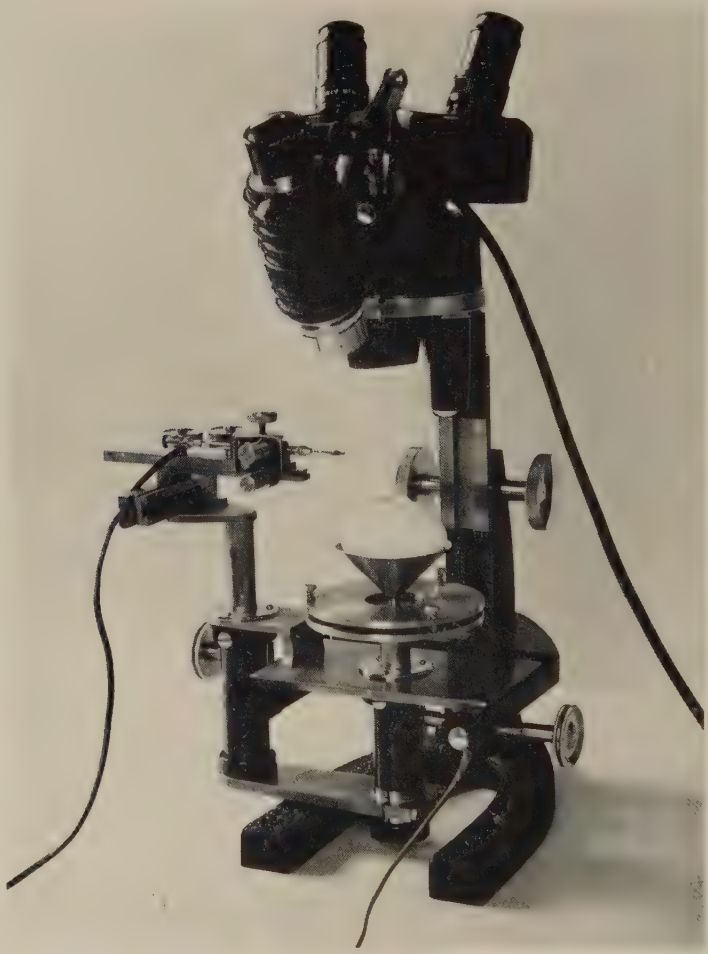

Fig. 2 Micromanipulator mounted on a binocular dissector ready for use.

during the operation on the embryo, (2) to permit the egg to be placed at any one of a wide range of angles which would bring the embryo in line with the opening in the egg, (3) to provide a means for bringing the embryo into the exact center of the visual and operative field, (4) to rotate the egg for an orientation of the longitudinal axis of the embryo in the operative field, (5) to permit the egg to be lowered or elevated and (6) to provide for the passage of an electric current through the egg thus eliminating the necessity for a second electrode in the albumin.

This egg-holder shown attached to the micromanipulator in figure 1 and shown in use in figure 2, consists of two heavy brass plates (3 mm. thick) circular in outline (92 mm. diameter). Between these two plates a similar disc but of smaller diameter (80 mm. diameter) is placed. This middle plate is held firmly by the pressure from the two outer discs, produced by the expansive force of three coil springs. A hole (31 mm. diameter) is drilled into the center of the top plate to allow an upright peg from the egg-shaped funnel to be screwed into the center of the smaller middle disc. By turning out the funnel, any other type of platform or holder may be turned in and manipulated in the same manner as the egg-holder. The egg-shaped metal egg-holder is of slightly larger dimensions than an egg to allow it to sink in somewhat. The friction of the rough egg-shell against the metal prevents slipping. Since the egg-holder is funnel-shaped in the vertical direction and egg-shaped in the horizontal plane, it will accommodate eggs of various shapes and sizes.

The contact surfaces of the three discs are polished and lubricated so that by pushing on the funnel's supporting peg, the middle disc will slip between the two larger discs with ease, firmness, and can be made to pass directly along any horizontal path through a radius of 13 mm. This is more than enough to cover the whole visual field under the low power of the dissecting binocular, and makes it possible to bring the embryo into the exact center of the visual field for best observation. To bring the embryo in line with the opening

in the shell, the egg may be tilted at various angles and firmly supported in the egg-holder at that angle. The egg may be elevated and lowered by operating the vertical rack and pinion of the micromanipulator. The whole egg-holder may be rotated on the supporting rod which fits into the upper end of the vertical rack and pinion. Thus the head end of the embryo may be made to face in any direction as desired for operative procedure. A drop of saline or strip of cotton moistened with saline placed at any point of contact between the egg and egg-holder will allow a current from the electrode in the egg to pass from the egg-substance through the membranes and shell and into the metal egg-cup which represents the other electrode. The saline passes through the pores of the egg-shell and establishes a connection between the egg contents and egg-cup for the passage of an electrical current. The detailed construction and operation of this egg-holder attachment for the micromanipulator may be understood from the key given below figure 1.

The author expresses his appreciation to Dr. M. F. Guyer and to Prof. G. Wagner for assistance in various ways in developing these instruments.

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THE DESIGN AND USE OF MICRO-ELECTRODES FOR THE PRODUCTION OF LESIONS IN THE PITUITARY RUDIMENT OF CHICK EMBRYOS

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THREE FIGURES

INTRODUCTION

Certain problems of interest to the author required a method for producing a limited and well-defined destruction of embryonic tissue which could be both accurately placed in a very small working space and precisely controlled in extent. One of these problems was to destroy the pituitary primordia in 33-hour chick embryos without injury to any other tissues. The elimination of the prospective pituitary areas by electrical means is here offered as a refined method for the hypophysectomy of early chick embryos.

Other workers have used different methods in their approach. Wolff and Stoll ('37), Ancel ('38) and Stoll ('39) used a method of irradiation by means of which they believed they could hypophysectomise chick embryos. Fugo and Witschi ('38), Fugo ('40) and Martindale ('41) used finely drawn black glass needles and excised the forebrain region of chick embryos (33–38 hours of incubation) as a method for hypophysectomy in these early stages.

It appeared that the removal of the forehead of chick embryos as mentioned above, constituted a rather drastic surgical procedure in the extent of the tissue removed, since the pituitary primordia represent a relatively small portion.

Such an extensive excision also involves the introduction of other variables which might play a part in the result, the obligatory removal of the primordium of the pineal gland being only one of these. Furthermore, such chick embryos could not develop an upper jaw, which would be essential in hatching. Fugo ('40) says that hypophysectomised embryos are not able to withdraw their yolk sacs at the end of the incubation period and found some living in the egg as long as 8 days beyond the normal incubation period without withdrawing the yolk sac.

The idea that a micro-electrode might be designed which would be small enough to be used in destroying the pituitary primordium appeared to have promise. At this point the author wishes to express his appreciation and thanks to Dr. R. K. Meyer for making this suggestion and for much help and interest in many ways in perfecting the electrodes and techniques of operation.

Electric currents of various types have been used by a number of investigators. Kopsch ('02) used electrocautery for studying determination in the chick blastoderm. Twiesselmann ('35) produced electrolytic lesions on the non-incubated chick blastodisc. Szepsenwol ('33) likewise made lesions in chick embryos by the use of electrolysis in his study of the organization centers of the auditory vesicle. Shorey ('09) used an electrocautery in a study of the effect of the destruction of peripheral areas (limb-buds) on the differentiation of neuroblasts. Hamburger ('34) however, claimed such a cautery to be unsatisfactory. Clark ('20) abandoned the electric cautery because it was difficult to localize a burn when the embryo was surrounded by fluid. E. C. Albritton (mentioned in Clark's paper) produced injuries by electrolysis, using a needle. The other lead was grounded to the albumin with a forceps; the source of current was a weak dry cell. Dantschakoff ('31) destroyed the prospective gonad region of 2-day chick embryos by electrocautery.

Horsley and Clarke ('08) worked out an extensive technique which is described in their paper dealing with the structure

and functions of the cerebellum of monkeys. They used iridioplatinum wire of various gauges from 21 (0.70 mm.) to 32 (0.19 mm.) in single and double glass tubes as unipolar and bipolar electrodes. After trying various types of injuries (anodal, cathodal, heat, spark gap, acids, bases) they found that the simple anodal electrolytic lesion was the most dependable. No surrounding injuries occurred and the necrosed tissue passed away. According to these authors, the principle involved in this tissue destruction consists in the formation of HCl from the NaCl and water in the tissues; the acid precipitates the protein of the cells, whereas in cathodal lesions an alkali is developed which likewise destroys the tissue but not so reliably.

It was this paper by Horsley and Clarke which suggested the principle basic to the methods and electrodes developed by the present author. Electrolysis and electrocoagulation has been refined to a microscale in the present investigation. The design of various new types of micro-electrodes, special methods for their construction, and their use in electrolysis and electrocoagulation are here described.

FASHIONING THE VARIOUS TYPES OF MICRO-ELECTRODES

To make the capillary tubes for the microelectrodes, the following procedure is used. Pieces of glass tubing (3 mm. diameter) are drawn out in a Bunsen flame as far as one can separate the arms. The portion drawn out into a capillary tube is broken into a number of pieces of about 8 cm. in length. These smaller pieces are then heated in the cooler flame of an alcohol lamp. They are again drawn out in a similar way and quickly, so that the resulting capillary tube loses its lumen at the mid-point along its length. A portion of this (about 6 cm.) is broken off so that the resulting piece has both an open and a closed end.

In order to make a double capillary tube, the method used by Horsley and Clarke is employed. First heat two lengths of glass tubing together so that they become fused at one point between the ends. Then by heating this spot till the

glass is soft, this joined point may be drawn out into a long and double capillary tube, the two tubes being united the one to the other throughout their length. The rest of the procedure is the same as for the single capillary tube.

In making the electrode, which must be as small as possible especially when working inside the neural tube of early chick embryos or attacking the pituitary primordium from the subcephalic pocket, it is essential not only to use extremely fine capillary tubes, but also very fine wire. Hence the smallest gauge of pure platinum wire available was used. It has a B & S gauge of 50 which is equivalent to 0.001 inch or 25 microns in diameter (bought from the J. Bishop & Co. Platinum Works, Malvern, Pa.). This fine wire is sharp enough to puncture through the vitelline membrane or to penetrate tissues such as the pituitary primordium when operating from the subcephalic pocket. Larger wires will not penetrate easily, but push the young embryo ahead of it.

The platinum wire comes on spools which cause it to be curly; it also has kinks here and there. It is very difficult to thread such fine wire into the capillary tube under these conditions. Passing the platinum wire through an alcohol flame, allowing it to heat to a glow, will remove the temper. If then pulled between two fingers, it will mould into a straight wire.

The wire is fed under a binocular into the larger open end of the capillary tube, which is held in the left hand between the thumb and index finger and rolled as the wire passes in. This rolling prevents the wire from adhering to the inner walls of the capillary tube. If the wire does stick, kinks form in it along the length of the wire outside, and cannot be fed in any farther, but must be removed, the wire smoothed and the operation repeated. When the wire appears at the closed end of the capillary tube where it becomes wedged at a point where the diameter of the inner lumen of the tube is about 25 microns, the tube beyond this point is cut off with fine scissors, and the wire pushed through the opening for a short distance.

To provide a sturdier wire at the base of the electrode for completing the circuit, a shorter piece of heavier platinum wire (B & S gauge of about 37) is fed in along the finer wire for a short distance where it soon becomes wedged and forms contact with the finer wire. If the fine wire alone were left here, it soon would break off from frequent bending. This base end of the electrode is fed into an alcohol flame so that it melts shut, thus holding the wire firmly in place. The superfluous free end of the finer wire may be cut off at this base.

To finish the electrode, it is necessary to cut off the excess fine wire that has appeared at the narrower end. The length of the free end of the electrode should be about 0.25 mm. Next, a point somewhere near the middle of the capillary tube is held near an alcohol flame and the threaded capillary tube is bent to make an angle of about 120 degrees included between the base and free end of the electrode. If the angle were reduced to 90 degrees, the light reflected from the elbow would produce a glare in the operative field. The fine end of the capillary tube may be placed near the flame and heated sufficiently to anneal the glass around the wire. This gives rigidity during operations and prevents liquids from rising in the tube, as well as facilitates its cleaning by presenting a smooth surface. The finished unipolar straight-tip electrode as described above is shown in figure 1.

To make the bipolar straight-tip electrode (fig. 1), the same operations as given above are repeated, except that a separate wire is fed into each bore of the double capillary tube.

In the fashioning of the unipolar hook-tip (glass-beaded) electrode (fig. 1), the operations outlined for the unipolar straight-tip electrode are repeated with some additions. When feeding the fine end of this electrode into the alcohol flame to anneal the glass around the wire, it is thrust progressively farther into the flame during which time the molten glass rounds up and slides back on the wire while still retaining continuity with the capillary shaft. This results in the formation of a small glass bead from the middle of which extends

the free end of the platinum wire. The excess length of the wire is then again cut off. Under a binocular, with the aid of a dissecting needle, the glass bead is again heated in an alcohol flame and bent upward to a vertical position, making an acute angle with the free end of the electrode (fig. 1). The bend should occur at the neck of the glass bead. Such a type of electrode is now small enough to be used in very small places (subcephalic pocket), permitting the operation on

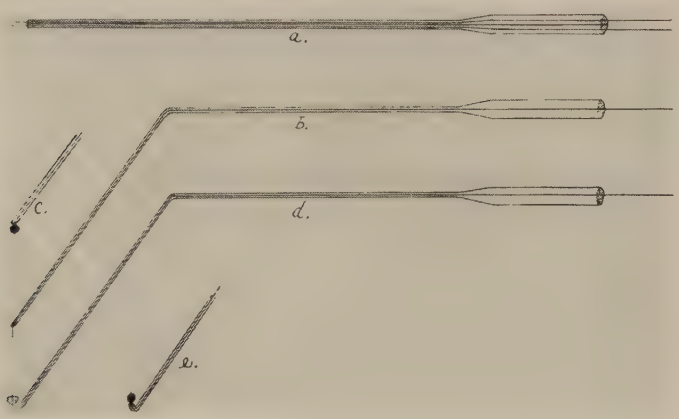

Fig. 1 Diagram showing construction of electrodes.

a. Bipolar straight-tip electrode.
b. Unipolar straight-tip electrode.
c. Unipolar straight-tip (platinum-beaded) electrode.
d. Unipolar hook-tip (glass-beaded) electrode.
e. Unipolar hook-tip (platinum-beaded) electrode.

embryonic structures (pituitary anlage) from below. The glass bead is of value in acting as a stop against the tissues, preventing too deep a penetration by the electrode proper.

It is necessary to sheathe these electrodes in these glass capillary tubes to provide the insulation necessary for preventing the formation of lesions at other places. And since the 50-gauge wire is so extremely fine, it requires a reenforcement to provide the rigidity requisite for thrusting into and withdrawing from embryonic tissues.

The measurements for the hook-tip (glass-beaded) electrode are given below. These dimensions serve in a general way for all the various designs of electrodes since they are built to the same scale.

Length of basal end of capillary tube	15 mm.
Largest diameter of bore at basal end of tube	1 mm.
Length of free end of capillary tube	17 mm.
Diameter of bore at free end of tube	25 microns
Outer diameter of free end of capillary tube	.15 mm.
Diameter of glass bead at free end of electrode	.25 mm.
Length of wire (B & S 50) extending from free end	.25 mm.
Length of wire (B & S 37) extending from basal end	50 mm.

In order to produce larger areas of tissue destruction where small spaces exist in which to work, wires of larger B & S gauge might appear to be useful. But such is not the case since large gauges of wire take up too much room and also are more difficult to bend in the flame after being threaded through capillary tubes of greater diameter. Therefore another type of electrode was designed and although it was fashioned with 50-gauge wire, it nevertheless has the advantage of larger gauges of wire at the lesion-producing end of the electrode.

This unipolar straight-tip (platinum-beaded) electrode is started like the other types. After the wire has been fed through the capillary tube the end projecting beyond is pulled out a distance of an inch or more. An abrupt kink is made in the middle of this free end and the wire folded back on itself. The point at which the bend forms is carefully fed into the flame of a small Bunsen burner. This high temperature causes the platinum to fuse and roll up, forming a platinum bead (fig. 1) which grows as it moves back, after the manner of the glass bead. The platinum bead may be of various diameters as desired. A platinum bead diameter of 0.25 mm. was found useful. Then by separating the two ends of wire on either side of the bead, and straightening these ends, we have completed a straight wire with a bead in the middle, all of this being of continuous platinum. The basal end of the wire is pulled back so that the platinum bead stops against the free end of the capillary tube. The tube is annealed against it to

add rigidity, and the surplus free end of the electrode, except for a length of about 0.25 mm. is cut off. This leaves a sharp platinum point on the platinum ball to help in the penetration of tissues. If no point but only the smooth ball is needed, as for insertion into the auditory vesicle, then the very end of the platinum wire is fed into the flame till a perfect sphere of suitable size results. From this point on the electrode is finished like the others. This completed electrode is shown in figure 1.

Sometimes this same type of electrode is needed for destroying larger areas of tissue from below the embryo. In such a case, the above described unipolar straight-tip (platinum-beaded) electrode may be modified into a hook-type, by bending the glass tube next to the platinum bead upward. We then have a platinum bead instead of a glass bead, and with or without the short end of the platinum wire extending from and above the ball, depending on how it is made (see description under the paragraph dealing with the unipolar straight-tip platinum-beaded electrode). This further modification may be designated as a unipolar hook-tip (platinum-beaded) electrode and is shown in figure 1.

It is understood that bipolar electrodes involving the features described for the various types of unipolars, can be made by the use of a double instead of a single capillary tube.

Sometimes electrocautery is a desirable method for destroying tissue. An electrode suitable for working in small spaces can be made by threading a double capillary tube, each part with a separate piece of wire. The tube is annealed around the two ends of wire that emerged from the fine bores, and the two ends gently twisted together between the fingers to form a spiral with many points of contact. The excess length is cut off. This twisted end makes a sufficient contact to complete the circuit, the wire becoming hot when a suitable amount of direct current is passed through. In completing the circuit, the two basal ends of the double electrode are inserted into the holes of separate insulated binding posts on the micromanipulator described elsewhere. To these same posts the two wires from the dry cells are anchored.

MOUNTING THE MICRO-ELECTRODES

The mounting of the electrode together with its grounding is diagrammatically shown in figure 2. Use is made of a rod and cylinder which come with the Chambers micromanipulator, but with the addition of a binding post on one end (fig. 3). The rod is tapered to fit into the tapered bore of the terminal cylinder. To mount the electrode, the coarse wire (37 gauge) at its base is fed into the narrower end of the cylinder until the annealed end of the capillary tube itself has entered for a short distance. Next a drop of hot sealing wax is placed where the glass tube entered the cylinder. When this cools

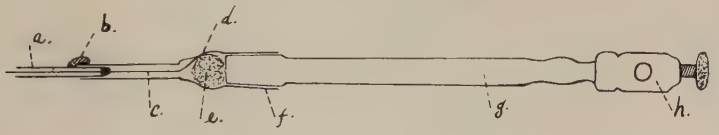

Fig. 2 Diagram showing mounting and grounding of electrode.

a. Capillary tube of electrode.
b. Drop of sealing wax.
c. Platinum wire at base of electrode.
d. Platinum electrode grounded against cylinder.
e. Cotton ball in position to hold wire in contact.
f. Chambers' metal cylinder.
g. Chambers' metal rod.
h. Binding post.

and hardens, it holds the electrode firmly and in the same horizontal line. To ground the basal end of the electrode, a small plug of cotton is thrust into the tapered bore of the cylinder to hold the wire firmly against the inner wall. The tapered end of the rod is then thrust into the tapered bore, achieving further contact for the conduction of the current. A binding post is threaded onto the other end of the rod to make a convenient contact for the wire coming from the source of current. To insulate this electrode mounting from the micromanipulator, a short piece of rubber tubing is slipped over it to the place where it is held in the thumb screw clamp of the micromanipulator.

USING THE MICRO-ELECTRODES TO PRODUCE LESIONS

It remains to describe how the electrodes are used in producing lesions. To do this, a type of operation will be chosen, such as destroying the pituitary primordia in 33-hour chick embryos from the subcephalic pocket. Use is made of the hook-tip (glass-beaded) electrode.

The electrode is mounted on the micromanipulator and the positive lead coming from three dry cells in series is fastened to the binding post. The other wire carrying the current away is bound to the micromanipulator stage. Formerly, a platinum cathode was placed in the albumen. To assure the passage of the current from the egg contents through the egg-shell and into the micromanipulator, a drop of saline is placed between the egg and egg-cup. In order to apply the electrode to the embryo from below, it is necessary first to rupture the vitelline membrane. This can be done by puncturing it in the proamnion region just in front of the head, with a mounted sewing needle which has been previously sterilized. Great care must be exercised to prevent the needle from going too far and rupturing the blastoderm. Such tears frequently result in the death of the embryo. An ear knife may also be used to slit the vitelline membrane. Fugo and Witschi ('38) and Fugo ('40) also warned against ruptures in the proamniotic membranes. These same authors and Martindale ('41) who followed their method, opened the vitelline membrane with finely drawn black glass needles.

When the vitelline membrane has been broken, it is pulled aside by the use of a finely drawn glass hook which is smooth (annealed) at the tip. It is pulled between the blastoderm and vitelline membrane to effect their separation.

The electrode is then placed directly in front of the head of the embryo by working the proper adjustments on the micromanipulator. The electrode is lowered to the bottom of the subcephalic space against the underlying blastoderm and then thrust under the head as far back as the posterior half of the forebrain. Following this, the electrode is raised upward until the point of the electrode can be seen to have

penetrated the stomodeal ectoderm and infundibular region of the neural tube, the free end of it extending slightly into the cavity of the third ventricle (fig. 3).

The lesion proper is produced by turning on the current. If the electrode is used as an anode, an acid precipitation of the cell proteins will occur. The tissues can be seen to turn white as a result, and some chlorine bubbles are released in the neural tube. The dye in the neighborhood of the electrode turns a brilliant red because of this acid condition. The extent of the lesion is controlled by observing the spread of

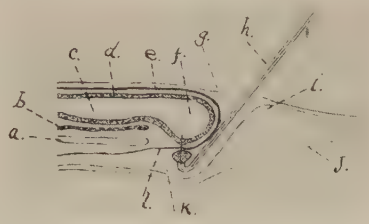

Fig. 3 Diagram showing hook-tip (glass-beaded) electrode in position for removing the pituitary primordium in a 33-hour embryo.

a. Foregut.
b. Notochord.
c. Mesocoele.
d. Mesencephalon.
e. Head ectoderm.
f. Prosocoele.
g. Ruptured vitelline membrane.
h. Unipolar hook-tip (glass-beaded) electrode.
i. Proamniotic fold.
j. Entoderm.
k. Subcephalic pocket.
l. Stomodeal ectoderm.

the white area and turning the current off at the proper time. The time required depends on the voltage used, but 3 to 4½ volts has been the usual strength required to drive the current through and produce a slow electrolysis. One can readily increase the voltage by the use of a resistance switch board to which the dry cells are connected. By moving the master switch, additional dry cells may be brought into or thrown out of the circuit. If the voltage is too high, bubbles form too rapidly, accumulate in the neural tube, and cause it to burst. It is best to use low voltage with only a fraction of a milleamp of current. It requires from a few seconds to about a minute

to produce a satisfactory lesion. The acid precipitation of the cell contents causes the tissue to adhere firmly to the electrode, and often results in this area of the neural tube and stomodeal ectoderm being pulled out. This removal of tissue produced the largest lesions and was of about the right extent to include the whole infundibular region.

To withdraw the electrode, some difficulty is encountered because of the adherence of the electrode to these tissues. A good way to release the head of the embryo from the electrode is to place a small glass spoon under the head from one side, press it against the electrode and lift the head off from the wire while lowering the electrode at the same time. The electrode may then be withdrawn in the same way as that in which it was placed into operative position.

The glass spoon may be fashioned by heating the end of a finely drawn glass rod to the melting point. By quickly squeezing the small molten ball with a fine forceps (cold), it can be flattened out and at the same time be bent at a right angle to the glass rod.

To clean the electrode of adhering tissue, it may be stroked with a camel's hair brush dipped in saline and then sterilized with another similar brush dipped in 70% alcohol. Sometimes it is necessary to remove the debris with a fine forceps.

LESIONS WITH A HIGH FREQUENCY CURRENT

At this point it would be well to introduce another and final method for the destruction of very small areas of embryonic tissues. This method was used in the latest experiments dealing with hypophysectomy in chick embryos and is regarded as being an extremely satisfactory and dependable way to destroy circumscribed areas of tissues to any desired extent. The results attained by the use of this technique greatly exceeded all expectations and are in general more satisfactory than those obtained by electrolysis.

The best anodal lesions were produced when sufficient tissue adhered to the electrode and was removed from the embryo. When tissue did not adhere, very frequently the lesion was

not extensive enough. However when enough chlorine bubbles accumulated, large lesions were produced at various places in the roof of the brain and in the eye vesicles. This of course is undesirable since there can be no control over the point or extent of the destruction when this happened. Whenever thin layers of tissues are involved, through which the electrode penetrates, electrolytic lesions are not as dependable as electrocoagulation, since they permit the escape of the lesion-producing ingredient to other regions. This fact accounts for the difference in the experience of the present author and that of Horsley and Clarke who thrust electrodes deep into thick layers of tissue (brain) so that the chlorine had no chance to escape except equally in all directions in the immediately surrounding tissue where the lesion was desired and invariably resulted. It seems that this latter method is superior to that of electrolysis even in heavy layers of tissue (involving deep penetration of the electrode) such as those with which Horsley and Clarke worked.

This improved method involves the use of a precision-made, spark-gap, high-frequency apparatus equipped with a 20,000-volt condenser and a positive micrometer control of power (minimum to maximum) for the finest type of electrocoagulation work either by desiccation or fulguration. In producing lesions, exactly the same set-up is used as before described, except for this change in the type and source of current together with the elimination of the second lead away from the egg which is not needed in this method. The wire running from this source of current is simply moored in the binding post of the electrode holder.

The high-frequency apparatus is manufactured by the General Automatic Corporation, Macedonia, Ohio, and may be purchased from any local surgical supply company (trade name of instrument, Driflash; price, $37.00). The instrument weighs but a few pounds and is portable.

Experience with this method has resulted in a number of observations which speak for its advantages. There is no release of bubbles of chlorine or hydrogen which may produce

ruptures and pressure distortions, or like chlorine, produce lesions elsewhere. One can always be absolutely certain that the desired area of tissue has actually been destroyed. The spread of the coagulating tissue can be observed in progress, evenly in all directions from the electrode, and its extent can be precisely controlled. There is no sticking of the tissues to the electrode; this makes possible an easy and quick removal of the electrode. It requires only a few seconds to produce the desired destruction with the micrometer control set to deliver the minimum amount of current. A 33-hour chick embryo may be hypophysectomised almost instantaneously, in just a few seconds. No second lead is necessary since the active electrode is the only one needed with this type of current. The heat precipitation of the cell contents by this electrical method can be carried out at any temperature from 65–100°C. During the electrocoagulation the temperature of the electrode does not rise; only the tissue surrounding the free end of the electrode suffers a rise in temperature because of its resistance to the current, and becomes coagulated. If the free end of the electrode is brought close to but not in actual contact with the tissue and the switch-button pressed, the current will jump the gap and coagulate the tissue directly below (fulguration) since the temperature of the tissue is raised to the coagulation temperature of protein. Fulguration is especially useful in work on blastoderms and where shallow destructions are wanted. This high-frequency coagulation is equally effective in working with either thin layers of tissues (early embryos) or thick structures (limb buds).

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REVERSIBILITY IN THE ORIENTATION OF THE CHICK EMBRYO AND THE QUESTION OF SITUS INVERSUS VISCERUM

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NINE TEXT FIGURES AND ONE PLATE (TWO FIGURES)

INTRODUCTION

There are two independent types of orientation of the early chick embryo in the egg. The first one is the orientation of the embryo relative to the shell-axes. It is usually indicated by the angle formed between the anterior-posterior axis of the embryo and the longer axis of the egg-shell. The other orientation refers to the surface of contact between embryo and yolk. This orientation is characterized by a transition from a symmetrical to an asymmetrical position which is initiated by a turning of the head. In the more frequent (normal) case the head turns to the right, and the embryo rests finally on its left side. In the rarer (abnormal) case the head turns to the left, and the embryo comes to lie on its right side. The terms "rotation" or "torsion" are also used, as synonymous with turning.

Both the turning of the embryo and its orientation relative to the shell-axes have been the starting-points for a series of problems.

Probably, K. E. von Baer (1828) was the first to express the idea that an abnormal turning of the chick embryo may be connected with the development of a situs inversus viscerum.

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He described the case of an embryo with abnormal rotation of the anterior part, and an inverse position of the heart ("Herz ganz umgekehrt gestellt"). He interpreted this condition as the beginning of a situs inversus.

About 50 years later Dareste (1877) discussed this question in a more detailed way. In his monograph on monstrosities one chapter deals with "l'inversion des viscères où hétérotaxie." Only such cases in which the heart shows an inversion of structure (not of position) should, according to him, be considered as "heterotaxia."

The term heterotaxia became a source of misunderstandings when it was applied indiscriminately to all cases of abnormal turning of the embryo. Warynski and Fol (1884) reported that they had produced abnormal turning of the embryo by unilateral heating. They emphasized that their "experimental heterotaxia" was not connected with any inversion of the structure of the heart. Yet, their paper has been quoted by several authors (Spemann and Falkenberg, '19; Morgan, '24, '27; Pernkopf, '26; Ludwig, '32) as more or less convincing evidence that situs inversus can be produced experimentally in chicks. Some authors took it for granted that abnormal turning is usually, or at least frequently, associated with situs inversus viscerum (Lillie, '27; Ludwig, '32). Others stated that they had not found situs inversus in chick embryos which had turned abnormally (Keibel and Abraham, '00; Witschi, '35).

While it has been rather common to connect abnormal turning with the idea of situs inversus, the abnormal orientation of the embryo relative to the long axis of the shell has rarely been viewed in this way. As far as I know, Bartelmez ('18) is the only author who has expressed the expectation that an embryo might have a situs inversus viscerum, if the anterior-posterior axis is "inverted," i.e., the head is opposite to the ordinary direction. Certainly, this idea is as much justified here as it is in the case of abnormal turning.

The undecided state of these problems is due to a technical difficulty. It is necessary to ascertain the two orientations in an early phase of development, and to study the left-right

asymmetry of the viscera in a later phase of the same embryo. This can only be done by observing the early embryos through transparent windows, and by dissecting (or sectioning) them when they have reached a sufficiently advanced stage.

All considerations concerning the possible connection between orientation of the embryo and situs inversus viscerum rest on the premise that the orientation of the embryo is irreversible. The literature referring to the question of reversibility is, however, very scarce. Since Rabaud ('08) the orientation of the anterior-posterior axis of an embryo relative to the long shell-axis has generally been considered as more or less stable. This author stated that, by means of windows, he could prove that the orientation did not change, "at least for the first five or six days of incubation." Changes of the direction of turning of the embryo (from the abnormal to the normal) were seen by Bremer ('24); he used mica-windows. The details of the changes were not described in Bremer's paper, as its main subject dealt with the position of the coeliac artery.

The purpose of the present investigation is to examine the question of reversibility of orientation and, if possible, to settle the relation between abnormal orientation and situs inversus viscerum. Transparent windows were applied in order to observe both the position relative to the long shell-axis and the direction of turning during several subsequent days. A number of those embryos of which the orientation had been recorded in this way reached advanced stages. By dissecting them the topography of the viscera could be ascertained.

METHODS

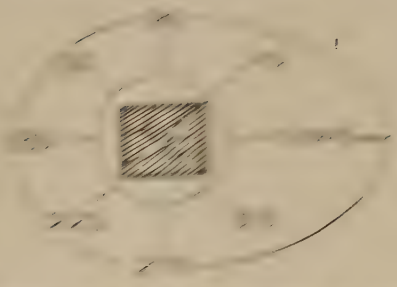
During the period from February to June 1942 transparent windows were made in 199 eggs which contained living embryos. Most of the eggs were at the end of the third day of incubation, the rest at the end of the second or fourth day. Six of the eggs were double-yolked.

The shell was opened at that part of the long side where the curvature is minimal (figs. 1, 10, 11). In most cases a

rectangular shell-window, about 12 × 11 mm. in size, was made by cutting the egg-shell with a carborundum disc run by a hand-motor, whereas the shell-membrane was cut with a sickle-shaped knife. Holding the egg horizontally and running the carborundum disc in a vertical plane seemed to cause hemorrhages. Probably concussions were transmitted to the area vasculosa which, by this method, was just under the disc. Therefore, in a second group, a less convenient but safer procedure was used. The rotating disc was kept fixed in a horizontal plane while the egg was turned and placed in four different positions relating to the sides of the window. In consequence of this procedure the embryo was every time as distant from the disc as possible. In both groups the window was covered with a mica-plate which was sealed to the shell by means of a wax-frame. In another group the technique of Bremer ('24, '26) was applied. Octagonal windows were cut in the egg-shell with a knife (scalpel). The shell-membrane was torn off with a forceps, and the cavity filled with sterile saline (0.7% NaCl). A piece of sterilized mica was placed on the opening, and the excess of saline removed to avoid the formation of air-bubbles. By means of heated beeswax the mica was sealed to the shell. In a small number of eggs the shell was cut with a knife, but no saline added.

Four quadrants were marked at the pointed end of the egg in order to record to what degree each egg had been rotated around its long axis. The eggs were rolled twice a day or more. Each egg was kept with the window down for at least 12 hours out of 24 hours. The hours of incubation mentioned in the text and tables are roughly indicative of the phase of development.

Sketches were made to record the position of the embryo relative to the long shell-axis, the turning of the embryo relative to the yolk, the place of the heart-beat, the general development of the embryo, and the main vitelline vessels. The figures utilized in the final text were redrawn by Miss Etta Piotti from the sketches prepared at the times of observation.



For the purpose of the present investigation it was sufficient to compare the incidence of survival following the different procedures of making windows. The best method was that which permitted the greatest number of embryos to develop beyond the twelfth day of total incubation. The results are

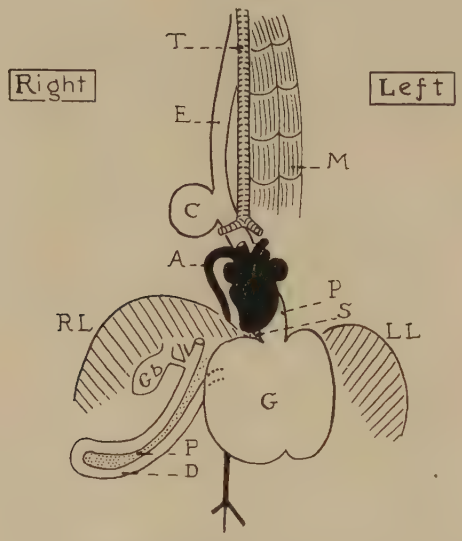


Fig. 2 Diagram of visceral topography showing the normal left-right asymmetry in chick embryos of advanced stages, and in hatched chicks. Ventral view. M, ventral muscles of the neck; T, trachea; E, esophagus; C, crop; A, aortic arch; Pr, proventriculus; G, gizzard; D, duodenum; P, pancreas; Gb, gall bladder; RL, right lobe of liver; LL, left lobe of liver; S, place of spleen dorsal of proventriculus.

presented in table 1. Embryos in double-yolked eggs (fig. 8) were not included in the table because of their poor survival.

The experiments are not numerous enough, and the factors involved too complex for statistical use; yet the table conveys a certain idea of the relative values of the different techniques. Evidently, the group "cutting with knife, no saline filling" is too small to permit any conclusion. But out of the three remaining groups the method "cutting with knife, saline filling" seems to be the best one.

TABLE 1

Relation between various techniques of making transparent windows and the number of embryos which continued developing (single-yolked eggs).

Technique	a	b	c		d	
	Total	Died before 12th day	Developed beyond 12th day		Hatched (included in c)	
			Absolute no.	Per cent of a [1]	Absolute no.	Per cent of a [1]
Carborundum disc vertical, no saline filling	74	67 (23)	7 (3)	9.5	0	0
Carborundum disc horizontal, no saline filling	82	59 (13)	23 (3)	28	2	2
Cutting with knife, no saline filling	5	2 (1)	3 (0)	60	1	20
Cutting with knife, saline filling	32	16 (3)	16 (2)	50	10	31
	193	144	49	25	13	7

[1] The percentages are given as a short-hand statement only, not as a basis for statistical evaluation.

Figures in brackets indicate how many of the embryos listed had hemorrhages in the area vasculosa.

2. Visibility

It was possible to observe the orientation of the embryos through the windows for 4 days in eight eggs, for 3 days in thirty-one eggs, and for 2 days in eighty-four eggs. All of these periods included the third day of incubation. In sixty-five eggs records of their orientation could be made during 1 day only which, in forty-eight cases, was the third day of incubation. In two eggs the embryos were seen on the fourth and fifth day only. After the fifth day the embryo is usually entirely covered by the membranes.

3. Axis-angles

The position of the embryos at the end of the third day was chosen as a basis for ascertaining the frequency of the different axis-angles. In twenty-seven eggs records of axis-angles were uncertain, or not available, at the end of the third day. The six double-yolked eggs were excluded as incomparable with the others. The remaining 166 eggs are recorded in table 2 according to frequency per octant, and in table 3 according to frequency per semicircles, top-quadrant

TABLE 2

Distribution of 166 embryos per octant at the end of the third day of incubation.

OCTANTS OF FIGURE 1	(a) ALL EMBRYOS	(b) DIED BEFORE 12TH DAY	(c) DEVELOPED BEYOND 12TH DAY	(d) HATCHED (INCLUDED IN c)
1°– 45°	10	8	2	1
46°– 90°	55	41	14	3
91°–135°	55	34	21	5
136°–180°	25	20	5	1
181°–225°	3	2	1	0
226°–270°	9	5	4	2
271°–315°	6	5	1	1
316°–360°	3	2	1	0
Totals	166	117	49	13

TABLE 3

Distribution of 166 embryos per semicircles and main quadrants at the end of the third day of incubation.

SEMICIRCLES AND QUADRANTS OF FIGURE 1	ALL EMBRYOS		DIED BEFORE 12TH DAY		DEVELOPED BEYOND 12TH DAY	
	No.	Per cent	No.	Per cent	No.	Per cent
Upper semicircle 1°–180°	145	87	103	62	42	25
Top quadrant 46°–135°	110	66	75	45	35	21
Lower semicircle 181°–360°	21	13	14	8	7	4
Bottom quadrant 226°–315°	15	9	10	6	5	3

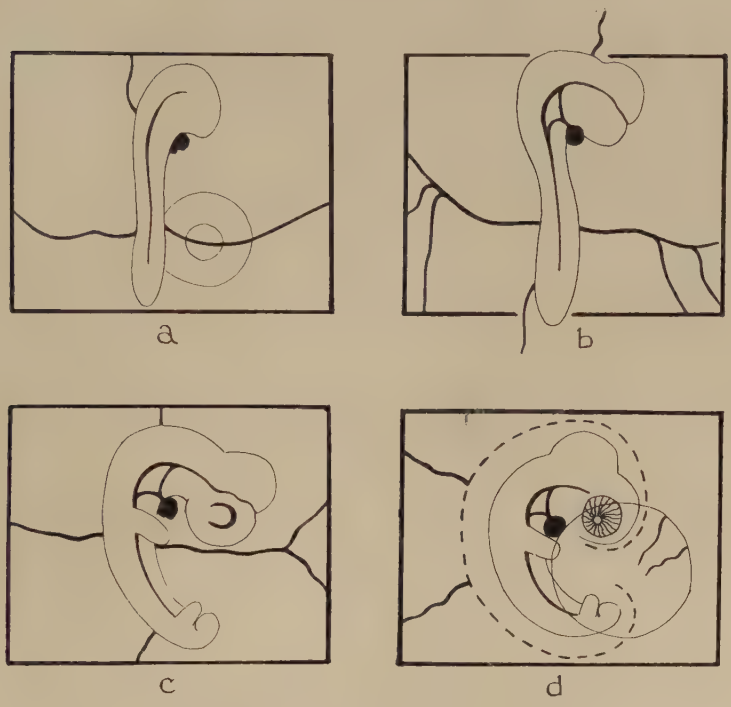


Fig. 3 Egg no. 357. Usual axis-angle (46°–90°). Normal turning. a, 53 hours. Turning of head has started. Nucleus of Pander still visible, posterior vitelline vein not yet formed. b, 71 hours. Turning of rostral parts advanced, posterior vitelline vein formed. c, 95 hours. Turning complete, ventral bend of head. d, 120 hours. Embryo floats in the amnion (interrupted line), but is still lying on its left side. Allantois well developed. On the twenty-first day a normal chick hatched.

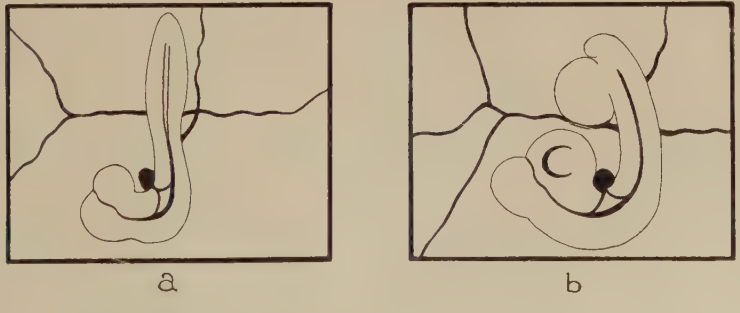


Fig. 4 Egg no. 385. Unusual axis-angle (226°–270°). Normal turning. a, 67 hours. b, 91 hours, cf. photograph figure 11.

and bottom-quadrant of figure 1. Embryos of more than 12 days are of special importance in the present study. Therefore those which died before the twelfth day and those which developed beyond the twelfth day are listed separately in both tables.

After the third day the ventral bending causes the embryo to assume a C-shape, but it is still possible to determine "the

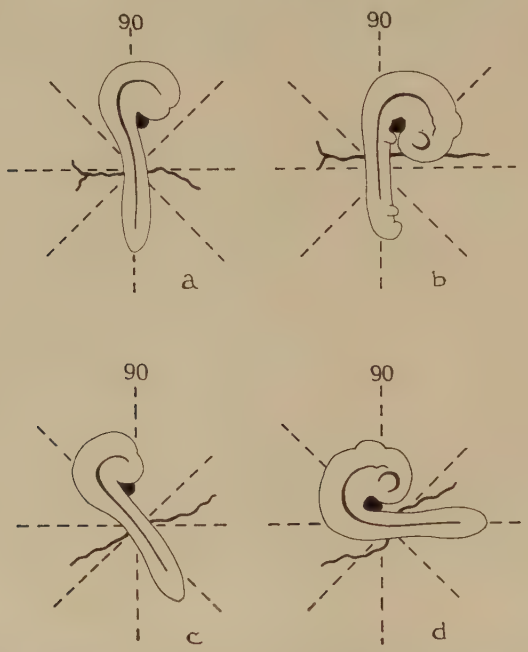


Fig. 5 Relation between ventral bend and axis-angle. a and b: position of head changed, axis-angle of caudal part unchanged (egg no. 172; a, 77 hours; b, 97 hours). c and d: position of head unchanged, axis-angle of caudal part changed (egg no. 139; c, 73 hours; d, 96 hours).

main direction" of the anterior-posterior axis of the embryo (figs. 3c and 4b). There are, however, cases with an unusually sharp bend forming an asymmetrical U (fig. 5 b and d). Here, the reading of the axis-angle will be more or less arbitrary. This difficulty was mainly encountered when changes in the axis-angle had to be recorded. Occasionally the vitelline vessels appeared to offer a frame of reference for

comparing different positions, and hence the position of the longitudinal axis is interpreted as being unchanged in the case of figure 5 a and b, but as changed in figure 5 c and d.

The 127 embryos which could be used for recording changes of the axis-angle are listed in table 4. Arbitrarily, a line of separation between significant and insignificant changes was drawn at 45°. Those fifty eggs which had been rolled 360°

TABLE 4

Occurrence of changes of axis-angles based on the observation of 127 embryos.

	NONE OR INSIGNIFICANT CHANGES		SIGNIFICANT CHANGES					
	Range		RANGE			DIRECTION		
	<10°	≥10° <45°	≥45° <90°	≥90° <180°	>180°	Clockwise	Counterclockwise	Clockwise and counterclockwise
Number of embryos	56	54	11	5	1	6	4	7
Totals	110		17					
Percentage	87		13					

immediately after sealing were distributed in the following way: none or insignificant changes forty-four (88%), significant changes six (12%). The ratio is the same as that of the totals (87% and 13%).

Out of seventeen significant changes there were four changes from the original to the other semicircle.

4. Abnormal turning

Out of 199 eggs the direction of turning could be stated with certainty in 190 eggs. In the group of 190 eggs abnormal turning occurred in nine embryos. The days during which the embryos were visible have been listed above.

The most important phenomena observed in these embryos were a repeated reversal of the direction of turning, a surprising speed at which a reversal may take place, and finally an

unexpected independence of the caudal part of the body in regard to the direction of turning. The last statement does not, of course, refer to the fact that the caudal part always turns later than the rostral part of the embryo. As seen in figures 6 c–e and 7 b, the allantois is not included in the abnormal turning of the caudal end of the body.

In six of the nine cases with abnormal turning it was possible to observe a series of subsequent phases which are summarized in table 5. Protocols which would have encumbered

TABLE 5

Changes in the direction of turning.

EGG	HOURS OF INCUBATION	TURNING		PLACE OF HEART-BEAT	REMARKS
		Head and rostral part of body	Caudal part of body		
No. 257	75	norm	O	r	Fig. 6 a
	93	abn	O	l	Kink, fig. 6 b
	95½	abn	abn	l	No kink, fig. 6 c
	126	norm	norm	r	Fig. 6 f
No. 260	76	norm	O	r	Similar to fig. 6 a
	76¼	abn	O	l	Kink, more rostral than on fig. 6 b
	93	norm	norm	r	No kink
No. 391	51	O	O	r	Development retarded
	67	abn	O	l	Figs. 7 a and 10
	90	abn	norm	l	Twist, fig. 7 b
	115	abn	abn	l	No twist
No. 241	75	norm	O	r	
	99	norm	norm	r	
	128	abn	norm	l	Twist, similar to fig. 7 b, except for opposite axis-angles
No. 185	73	norm	O	r	
	96	abn	abn	l	
	120	norm	norm	r	
No. 63	70	norm	O	r	
	74	abn	abn ?	l	
No. 126B	89⅓	norm	norm	r	Fig. 8 b
	90¾	abn	abn	l	Fig. 8 c

norm = normal, abn = abnormal, O = no turning yet, r = right side, l = left side.

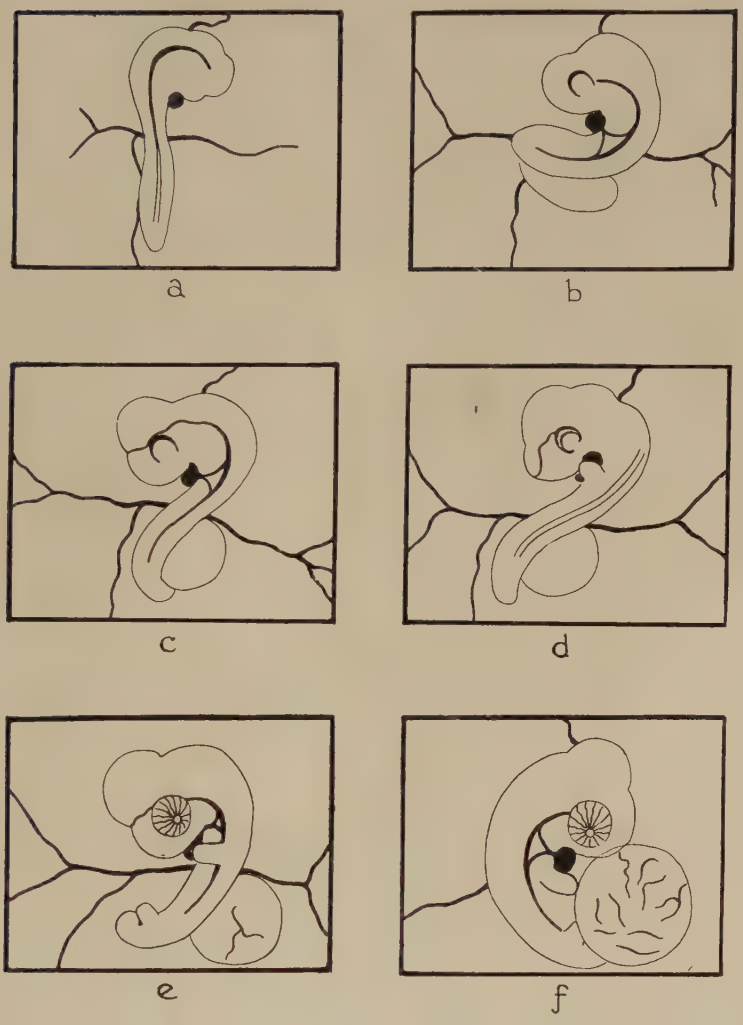


Fig. 6 Egg no. 257. Usual axis-angle (46°–90°). Turning from normal to abnormal, and back to normal, with temporary kink. a, 75 hours. Normal turning of the head. b, 93 hours. Head and rostral part of body lying on their abnormal (right) side, kink of caudal part. c, 95 hours 30′. Kink has disappeared. Abnormal turning complete, except for tail and allantois. d, 99 hours. Abnormal turning maintained, heart-beat protruding on left side. Caudal part of body, to some extent, seen in dorsal view. e, 116 hours 20′. Complete abnormal turning, inclusive of leg-bud and tail, but exclusive of allantois. f, 126 hours. Head and body lying on their left (normal) side now.

the table are given as supplementary notes. The term kink found in the table is used if the anterior-posterior axis forms an acute angle instead of being either straight or curved. The term twist refers to differences between the frontal planes of two apposite parts exceeding 90°.

A tangible idea of the present observations will be obtained by a combined use of table 5, the supplementary notes, and the figures 6, 7, and 8.

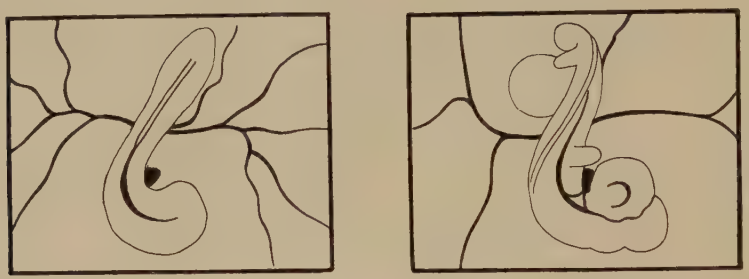


Fig. 7 Egg no. 391. Unusual axis-angle (226°–270°). Abnormal turning with temporary twist. a, 67 hours. Head and rostral part of body lying on their right (abnormal) side; no turning of caudal part yet; cf. photograph figure 10. b, 90 hours. Caudal part has turned on its left (normal) side. Left wing-bud and right leg-bud facing the observer: 180° twist. Allantois ventral of caudal part of body. For later development see text.

Supplementary notes to table 5. Egg no. 257. Window made at 75 hours; egg rolled 360° around the long shell-axis: no immediate change of orientation. Completely abnormal position during at least 20 hours. After return to normal position apparently regular development; but at 154 hours embryo found dead. — Egg no. 260. Window made at 76 hours; egg rolled twice 360°: immediate change of turning at 76¼ hours as recorded in table 5. Rolling of the egg 360° for a third time: no new change. Kink persisted at least up to 80 hours. — Egg no. 391. Window made at 51 hours; egg rolled 360°: no change of orientation. At 67 hours the retardation of development mentioned in the table is overcome. At 139 hours amnion well developed, embryo presenting ventral and right-side view; normal circulation in allantoic vessels. But at 148 hours embryo dead. — Egg no. 241. Window made at 75 hours; egg rolled 360°. Turning remained normal for at least 24

hours. When the twist was discovered at 128 hours, the embryo was dead. — Egg no. 185. Window made at 73 hours; egg rolled. After this both the axis-angle and the sense of turning were found unchanged, although the area vasculosa had temporarily been caught on the edge of the shell-window. Later changes listed in table 5. Abnormal turning persisted at least until 101 hours. At 120 hours the embryo presented a complete right-side view. On the twenty-first day the chick peeped and pecked, but died before hatching. — Egg no. 63.

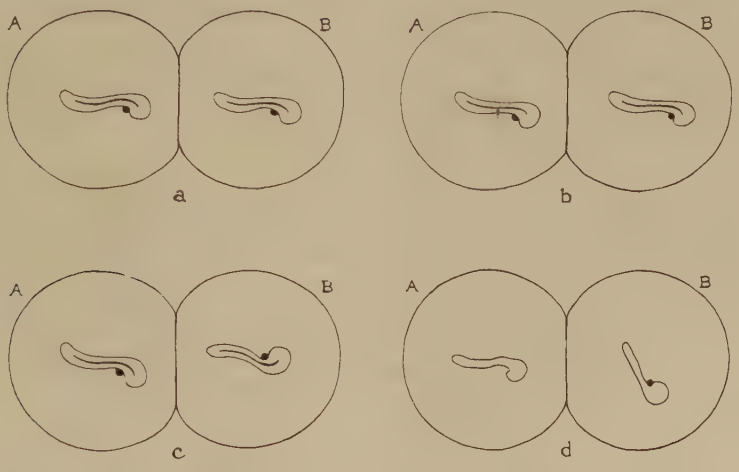


Fig. 8 Egg no. 126. Double-yolked egg with two embryos. Embryo A near the broad end, embryo B near the pointed end of the egg (vitelline vessels omitted). a, 67 hours. A and B turned normally. b, 89 hours 23 minutes. A and B unchanged. c, 90 hours 46 minutes. A unchanged, B turned to the abnormal position. d, 94 hours. A dead, B alive, but anemic. Note: the change of axis-angle in B is not certain. B was found dead at 112 hours.

Window made at 66 hours: head and rostral part of body in position of normal turning. After rolling the egg 360° conditions unchanged, at least until 70 hours. Later changes listed in table 5. Embryo found dead after 114 hours, in abnormal position. — Egg no. 126, embryo B. Double-yolked egg, slightly larger than an ordinary egg. Window made at 67 hours. Conditions found and further development appear from figure 8 a–d.

Embryos not listed in table 5. Egg no. 225. Opened at 76 hours: living embryo lying with its right (abnormal) side on the yolk. After the window had been sealed, the area

vasculosa was caught on the edge of the shell. When the egg was rolled, the blastoderm became detached from the yolk, and the embryo died. — Egg no. 114. Opened at 43 hours; records of this period incomplete. At 73 hours embryo found lying on the right (abnormal) side with heart beating on the left. At 94 hours embryo dead and shrivelled. No statement concerning the position possible.

Methods used for making the windows in the nine cases of abnormal turning. Carborundum disc vertical, no saline filling: nos. 63, 114 and 126. Carborundum disc horizontal, no saline filling: nos. 185, 225, 241, 257 and 260. Cutting with knife, saline filling: no. 391. Small hemorrhages were observed in nos. 225 and 257.

5. Dissections

In a total of 193 single-yolked eggs which had been supplied with windows, forty-nine embryos developed beyond the twelfth day, including thirteen chicks which hatched. The forty-nine birds were dissected and showed a normal asymmetry of the viscera (diagram fig. 2), no matter whether their axis-angle, or direction of turning, had been normal or abnormal. In one case the gall bladder was lacking. None of the six double-yolked eggs reached the twelfth day. In addition fourteen chicks which had hatched from eggs without windows were dissected: their situs viscerum was normal.

DISCUSSION

The axis-angle and the direction of turning are independent of each other. This appears from the fact that the four possible combinations between normal or abnormal axis-angle and normal or abnormal turning were actually observed in the present series, as it is shown in figures 3, 4, 6 and 7.

1. Reversibility of orientation

Both the axis-angle and the direction of turning proved to be less stable than it had been assumed.

The traditional idea that the axis-angle "does not change during the first 5 or 6 days of incubation" is based on a

statement of Rabaud ('08), as mentioned in the introduction. Rabaud, however, did not indicate the type of his windows nor the number of his observations. Moreover, it is difficult to read the axis-angle on the first day (Kopsch, '27); on the other hand, the readings become uncertain on the fifth or sixth day, due to the movements of the embryo in the amnion. The doubtful readings having been eliminated, I found changes of axis-angles amounting to 45° or more in seventeen cases, inclusive of six cases with changes of more than 90° (table 4). Seven of the seventeen cases showed successive changes in opposite directions.

Turning of the head from one direction to the opposite was recorded in six embryos. Three of them turned from the normal to the abnormal, and back to the normal position. In a seventh case the head turned in the abnormal direction and stayed there, while the caudal part of the body turned first to the normal, and then to the abnormal side. Bremer ('24) had observed that all embryos, which he found abnormally turned on the third day, changed to the normal, provided they were still alive during the fourth day. In my series one head remained permanently in the abnormal position, while another head turned abnormally after the fourth day. Apparently, any embryo which is seen in the abnormal position at the end of the third day, may have turned in the normal direction previously, and has a certain chance to turn to the normal position, if development continues.

There is no reason to assume that my material was peculiar in any sense. Irrespective of changes in the same egg, the distribution of axis-angles in my series was similar to that observed by Rabaud and most other authors (cf. the review by Bartelmez, '18; Kopsch, '27). The frequency of abnormal turning in my material was also of the order of magnitude reported by other investigators. On the third day of incubation abnormal turning was seen in 3% by Féré ('00), probably in more than 5% by Bremer ('24), and in 6% by Gray, Worthing et al. ('40, '42). Neglecting the differences of days, I can state that in my series of 190 embryos nine, or 5%, showed

abnormal turning. Four of them, or 2%, were in the abnormal position at the end of the third day.

During the first 3 days of incubation the position of the anterior-posterior axis is a matter of the embryo as a whole. But, after the third day, with the development of the ventral bend, the position of the head and that of the caudal part of the body become, to some extent, independent. The difficulty to ascertain which part moved and which remained in its place has been discussed above with reference to figure 5.

Independent turning of parts of the embryo has been known for a long time. Dareste (1877), and especially Bremer ('24), described how the turning of the heart is occasionally dissociated from the turning of the head. In the present series of observations it was mainly the caudal part of the body which showed a striking independence. In four cases the direction of turning of the caudal part was temporarily opposite to that of the rostral part. The kinks and twists which can result from such dissociation were also known before; even their reversibility had been anticipated by Keibel and Abraham ('00, their fig. 36). But the actual formation and disappearance of kinks and twists could, of course, not be observed without transparent windows.

What mechanisms are responsible for the turning of the embryo? The possible mechanisms can be classified into three groups: unequal growth, "locomotion," and external forces. The term locomotion, although far from being satisfactory here, is adopted from the behavioristic school (cf. Orr and Windle, '34).

In order to ascertain the role of unequal growth one could try to determine the earliest left-right differences of mitotic coefficient, rate of cell-death, and cell-displacement.

Coghill ('29) described a sudden reversal of coiling in Amblystoma embryos and interpreted it as a forerunner of locomotion (swimming). It seemed to me that the turning of the chick embryo might be comparable to the coiling of the Amblystoma embryo, as I was impressed by the reversibility and occasional speed of the turning. With the help of

Dr. R. S. Morison an attempt was made to stimulate 3- and 4-day-old embryos electrically, in order to make them turn around their long axis. The results were negative, because there is no reaction to electrical stimuli before the sixth day, as we found in agreement with the statements of other authors (Kuo, '32; Orr and Windle, '34).

External forces controlling the turning might be located in the colloidal media apposite to the embryo, namely, the egg-white, the vitelline membrane, and the yolk. Doctor Bremer informs me that in alligator eggs no turning of the embryo is seen, because their soft yolk does not resist the ventral bending. After the third day, when the amnion is closed, contractions of this membrane might also play a role. Bremer ('24, '26) turned the heads of chick embryos in the abnormal direction by operation, but they returned to the normal position after some time, although the amnion had been cut open (cf., however, Vintemberger, '27, p. 453).

Recently, Gray, Worthing et al. ('40, '42) injected various optically active substances under the blastoderm before the beginning of the turning. Many of these substances increased the rate of abnormal turning. The authors concluded that the reactivity to the substances injected is different on the right and left side of the embryo. As far as I can tell from the preliminary reports, this conclusion is inevitable. Gray, Worthing et al. closed the shell-window with a shell-fragment, recorded the direction of turning at 72 hours of incubation, and fixed the embryos subsequently (personal communication). If the injection of agents which they utilized could be combined with continuous observation through transparent windows, more light might be thrown on the mechanisms of turning.

The changes of axis-angles in an individual egg may be due to (a) displacement of the embryo relative to the area vasculosa, (b) displacement of the area vasculosa relative to the yolk, and (c) movements of the yolk relative to the shell. It might be possible to investigate (a) and (b) by tagging different spots of the egg-white (Ferret and Weber, '04) and

yolk, and by observing displacements of these landmarks through several transparent windows. Unequal growth, "locomotion," or external forces may be the responsible mechanisms here as in the turning of the embryo. Movement of the yolk (c) is mainly controlled by the chalazae. Bartelmez ('18) pointed out that the chalazal axis is not necessarily identical with the long shell-axis, and that this relation should be investigated in the hen's egg as carefully as it had been done in the pigeon's egg.

2. Relation between abnormal orientation and situs inversus viscerum

Since the orientation of the embryo proves to have a considerable degree of reversibility, any connection between abnormal orientation and situs inversus viscerum becomes unlikely.

In the present series the topography of viscera could be studied in seven embryos which were orientated in the abnormal semicircle, and in one embryo which was in the position of abnormal turning at least from the ninety-sixth to the one hundred and first hour. All of these showed a normal right-left asymmetry of the viscera. In the forty-one embryos and chicks which had been normally oriented, and in fourteen control chicks, the topography of the viscera was equally normal.

Upon attempting to find out how frequently situs inversus occurs in fowl, I made a strange discovery. Dareste (1891, p. 346) stated that he had seen inversions of viscera in "chick embryos of a certain age," the gizzard being on the right side. But there was no published reference to any definite case of situs inversus, based upon actual dissection of a hen, rooster, or other fowl. Dr. R. M. Strong was so kind as to tell me that the topic of situs inversus is not in the index of volume 3 of his "Bibliography of birds" now in preparation. On personal inquiry, a number of investigators informed me that they had never seen a situs inversus viscerum in adult fowl, hatched chicks, or chick embryos in advanced

stages, and that they did not know of any references. I am greatly indebted to Drs. E. A. Boyden, J. L. Bremer, Walter Landauer, F. T. Lewis, B. M. Patten, Oscar Riddle, and A. R. Romanoff for this information. Quite recently, a paper by P. Gruenwald ('42) appeared dealing with the asymmetry of the müllerian ducts. He briefly mentions a chick embryo of 5 days 19 hours with a situs inversus of the abdominal viscera. Doctor Gruenwald was kind enough to lend me his serial sections. They show indeed an inversion of liver and gizzard, as there is no reason for assuming a technical error. The thoracic viscera, of which no sections were available, were presumably also inverted, as Doctor Bremer concluded from the position of the coeliac artery.

A statement of complete situs inversus should, of course, include the topography of the cervical viscera. Böker ('31) emphasized that, in chicks, the shift of the trachea and esophagus from a median position to the right side occurs during the second half of embryonic life. I found the trachea still in the median position in a 16-day embryo, and this is one of the reasons why I postponed the dissections until after the twelfth day.

Confusion has been caused by applying the concept of situs inversus viscerum to early phases of chick embryos, in which the heart is the only asymmetrical organ. As mentioned in the introduction, Dareste had restricted the term "heterotaxia" to embryos with "at least" an inversion of the heart. It appears from Dareste's diagrams (1891, p. 350) that his "inversion of the heart" is a cardiac loop coiled in an abnormal (dextral) sense. Whether or not he had actually observed any hearts of this type is difficult to tell from his book. I have not succeeded in finding any record of a dextral cardiac loop. Dr. B. M. Patten informs me that he recollected one instance of a dextral cardiac loop, and Dr. J. L. Bremer tells me that he has never seen such a case.

There are some inconsistencies in the use of the terms dextral and sinistral (D'Arcy W. Thompson, '42, pp. 820 and 881). The best way is to refer the terms to a screw, as it is

done in modern physics and chemistry. A screw is dextral if it is forced into a piece of wood by turning the screw-driver clockwise. In the opposite case the screw is sinistral. A coil should always be considered as a part of a screw. In figure 9 the embryos a and b were drawn after diagrams of Bremer ('24, p. 235) in which all of the heart-loops are coiled sinistrally; I have added diagram c in order to give a clear idea of a dextral (abnormal) coiling.

The relation between the formation of the cardiac loop and the turning of the embryo is a special problem (Patten, '22).

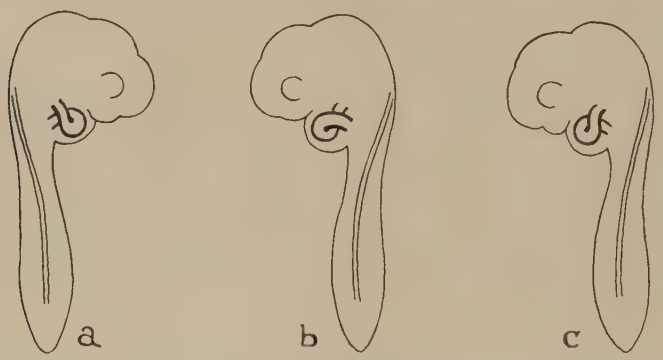


Fig. 9 Diagram of chick embryos during the first part of the third day of incubation. Dorsal views. a, head and heart turned to the right (normal), coiling of cardiac loop sinistral (normal); b, head and heart turned to the left (abnormal), coiling of cardiac loop sinistral (normal); c, head and heart turned to the left (abnormal), coiling of cardiac loop dextral (abnormal).

Therefore considerations of the possible connection of situs inversus viscerum and abnormal orientation should not depend on the heart only, and the question cannot be solved by sectioning early embryos.

The problem could conceivably be settled by combining window-observations with subsesquent dissections on a much larger scale than in the present study. What number of window-eggs would be necessary for demonstrating that situs inversus is more frequently associated with abnormal than with normal orientation of the embryo? My estimate will be based on observations combined with a number of assumptions.

It is reasonable to assume that the frequency of situs inversus is not more than 1 in 4000 chicks, irrespective of abnormal and normal orientation of the embryo. Let us suppose the result, that three times as many cases of situs inversus are observed in abnormal as in normal embryos, would be significant. Finally, let us lower the standards for establishing the presence of complete situs inversus, and dissect (or section) the embryos on the sixth day of incubation.

If an egg is supplied with a mica-window on the third day, the chance of reaching the sixth day should be two-thirds for embryos with normal orientation. Embryos with abnormal axis-angles have the same chance of development (cf. tables 2 and 3). Embryos with abnormal turning have a very small chance to reach the twelfth day; but it is permissible to consider their chance of reaching the sixth day also as two-thirds (cf. supplementary notes to table 5).

Let us assume three cases of situs inversus are required in each abnormal group. The frequency of abnormal axis-angles is about one-eighth (table 2). If we were to supply 60,000 eggs with windows on the third day, 52,500 of them would be normal, and 7500 would be abnormal, while on the sixth day 35,000 would be normal and 5000 abnormal. If there are three cases of situs inversus in the 5000 abnormal embryos, and seven cases in the 35,000 normal embryos, the frequency of "Abnormal : Normal" will be 3 : 1, as postulated. Let us assume that one investigator can take care of sixty window-eggs per week, inclusive of operation, observation, dissecting (sectioning) etc. Then, 60,000 eggs could be done by ten workers in 2 years.

For obtaining three cases of situs inversus in embryos with abnormal turning 150,000 window-eggs are needed on the third day, since the frequency of abnormal turning is only one-twentieth. Suppose one of the most potent agents used by Gray, Worthing et al. would increase the rate of abnormal turning by three times, without decreasing the chance of reaching the sixth day. Then, 52,500 window-eggs would be necessary on the third day.

All these calculations would need a statistical correction for rare events. In other words, the number of eggs is still too small. These considerations will illustrate that the time and expense of such an investigation would not be justified by eventually finding a certain correlation between abnormal orientation and situs inversus viscerum. Moreover, reversible factors are involved in the abnormal turning. Therefore its relation to situs inversus can hardly be settled satisfactorily by statistical methods.

I acknowledge my indebtedness to Dr. G. B. Wislocki who most generously provided me with the facilities of his laboratory. I desire also to thank him and other members of the Department of Anatomy for many valuable suggestions.

SUMMARY

1. The position of the anterior-posterior axis of the chick embryo relative to the long shell-axis (axis-angle) and the direction of turning of the embryo on the yolk were observed through mica-windows during several subsequent days. Embryos which developed beyond the twelfth day of incubation were dissected.

2. The left-right asymmetry of the cervical, thoracic, and abdominal viscera was normal in all cases, inclusive of seven embryos with abnormal axis-angles (head in the unusual semicircle) and one embryo with temporarily abnormal turning (right side on the yolk).

3. Reversible and irreversible changes of axis-angles were observed in a number of cases. Abnormal turning always involved reversible phenomena. Thus turning from the normal to the abnormal, and back to the normal, was seen in three embryos.

4. Independent turnings of the caudal and rostral parts of the embryo (temporary kinks and twists) were conspicuous; independent displacement of the two parts was less pronounced in changes of the axis-angle.

5. Mechanisms which may control the changes of orientation are discussed. Since the orientation of the embryo proves to possess a high degree of reversibility, causal relations between abnormal orientation and situs inversus viscerum become less likely. A statistical investigation of these problems would require time and expense which would be out of proportion with the possible results.

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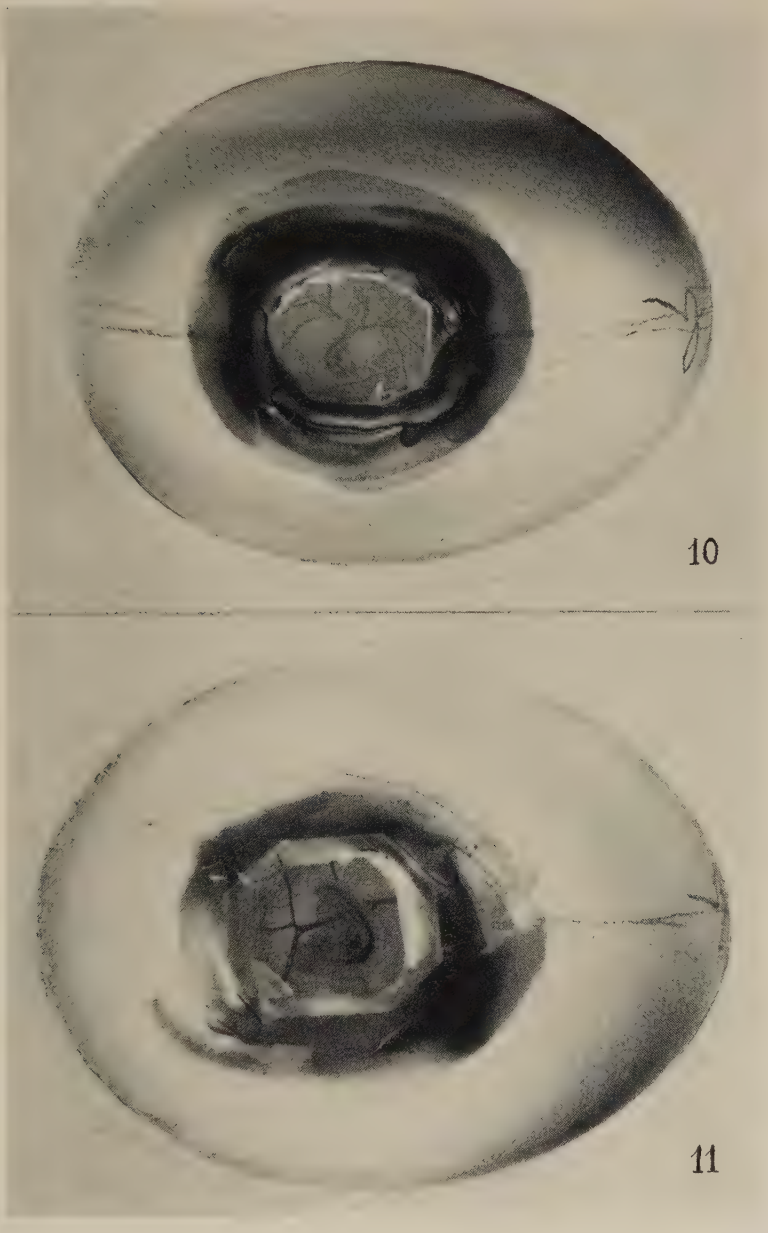
PLATE 1

EXPLANATION OF FIGURES

10 Egg no. 391 (cf. fig. 7 a). Photograph taken at about 67 hours of incubation. Unusual axis-angle. Abnormal turning of head and rostral part of body; caudal part not turned yet. × 1½.

11 Egg no. 385 (cf. fig. 4 b). Photograph taken at about 91 hours of incubation. Unusual axis-angle. Normal turning of head and whole body. × 1½.

NOTE: Retouch has been applied to the outlines of the eggs, but not to the structures seen in the windows.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STUDIES OF THE AVIAN PITUITARY

I. THE DEVELOPMENT OF THE DUCK PITUITARY WITH SPECIAL REFERENCE TO CHANGES IN THE PARS BUCCALIS

BEN THOMAS PAINTER

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TWO PLATES (ELEVEN FIGURES)

INTRODUCTION

The only adequate account of the cellular differentiation arising during the morphogenesis of the pre- and and post-natal avian pituitary is that of Rahn ('39). While Atwell and Sitler ('18), and Atwell ('39) have described the embryonic development of the chick pituitary, they have confined themselves entirely to its anatomical changes.

The adult duck pituitary has received only slight attention in the comparative studies of Stendell ('14), and deBeer ('26). Pfeiffer ('25), deBeer ('26), Lups ('29), and Friedman ('34) have given brief accounts of its anatomy during the early stages of incubation. The work of Rahn and Painter ('41) summarizes the outstanding features of the adult pituitary of both the duck and the chicken and points out, after comparison with other avian types, the uniformity in morphological structure of the pars buccalis of the bird.

In view of the relatively few studies made of the avian pituitary the writer considers it of value to give a more detailed account of the morphological, especially the cytological changes that occur in the duck pituitary from 9 days of incubation to the adult condition. Particular attention is paid to the first appearance of the basophiles and acidophiles, to the time of disappearance of the residual lumen and epithelial stalk, to the composition of the two cytologically different

zones of the pars anterior, and to the question of the presence of a pars intermedia. A preliminary account of this study was reported by Painter ('38).

MATERIAL AND METHODS

The material used for all stages of this study was obtained from the White Peking duck, Anas boschas.[1] Thirty-two glands constituted the embryonic series from the ninth day of incubation to hatching age, thirty-one from hatching to 3 months of age, and forty from 4 months to 20½ months of age.

In order to retain the proper relation of the pituitary gland to the brain, a few of the embryonic glands were left intact in the sella turcica. Those used for study of early morphological stages were fixed in Bouin's, decalcified when necessary, serially cut in transverse or sagittal plane at 8 μ, and stained in Ehrlich's hematoxylin and eosin. Most of the other glands, both pre- and post-natal, were fixed in either Dawson's (Dawson and Friedgood, '38) or Helly-osmic fluid. For comparative purposes a few were fixed in Severinghaus's ('32) or Helly's fluid. These latter two, however, gave an unsatisfactory fixation.

All glands for cytological studies were sectioned in a sagittal plane at 4 μ and stained according to "a slight modification of Heidenhain's "Azan" modification of Mallory's triple connective tissue stain" (Dawson and Friedgood, '38).

OBSERVATIONS

I. Morphogenesis of the pituitary

Nine-day embryo. Although the pituitary is directed anteroventrally to the point where it makes connection with the epithelial stalk,[2] its entire dorsal surface is closely applied to the surface of the tuber cinereum, with only a thin sheet of connective tissue between the surfaces (fig. 2). The infundibular

[1] The author is indebted to Mr. Brooks Farrar of South Easton, Massachusetts, for his cooperation in obtaining some of the older material for this study.

[2] Called "hypophyseal stalk" by Atwell and Sitler ('18), and Rahn ('39); "epithelial stalk" by Atwell ('39).

outgrowth, the distal portion of which is destined to become the pars nervosa, shows only slight development. Other sections of the gland show the residual lumen extending almost to the epithelial stalk. Except for occasional suggestions of a lumen, the stalk is solid, and extends from the anterior ventral end of the gland to the roof of the mouth. The two diverticula from the base of the stalk may be peculiar to this particular gland. The posterior and dorsal diverticulum, however, may represent the "dorsaler Fortsatz" of Lups ('29).

Ten-day embryo. As seen in figure 1, the residual lumen extends nearly the full length of the gland. Figure 5 shows the epithelial stalk still connected with the roof of the mouth. The knob of cells projecting posteriorly from the base of the stalk is probably the remains of Seessel's pouch. Here, as in the 9-day embryo, the infundibular process shows no outgrowths or diverticula.

Both the pituitary gland and the tuber cinereum have assumed a more horizontal position. The caudal portion of the pars buccalis is so close to the infundibular process that no connective tissue can be detected between them. The cephalic portion, although arranged in the same general axis as the caudal portion, no longer touches the tuber cinereum.

The relations of the caudal and cephalic portions of the pars buccalis of the duck to each other and to the tuber cinereum are quite different from the corresponding relations in the early chick pituitary.[3] Atwell ('39) reports that in the 7-day embryonic chick gland (which corresponds in developmental stage to the 10-day duck gland) "the caudal portion of the gland lies parallel with the neural lobe and the brain floor while the rostral portion is arranged in the direction of the epithelial stalk. The two portions thus make an angle of approximately 90° with each other." Rahn ('39) reports this same condition in his description of the 6- and 9-day chick embryonic glands.

Eleven-day embryo. Although the infundibular process has undergone no apparent change, the pars buccalis is somewhat

[3] The incubation period in the duck is 30 days; that in the chick is only 21.

more compact, especially in the posterior end. The residual lumen is less extensive. The epithelial stalk is solid and no longer extends to the roof of the mouth (fig. 3).

Twelve-day embryo. The greatest change that has occurred during the eleventh and twelfth day stages is the increase in the size of the pars buccalis, particularly in the cephalic portion. The infundibular process reveals no protuberances at this stage, but it is more pronounced and assumes a position characteristic of that of the adult gland. Little or no change has occurred in either the epithelial stalk or residual lumen since the eleventh day (fig. 4).

Thirteen-day embryo. Of great interest here are the tubular epithelial strands which lie perpendicular to both the floor of the brain and the residual lumen (fig. 9). They are separated by mesenchymatous strands and numerous blood vessels. Other sections of the gland reveal within these cords numerous lumina which are particularly conspicuous near the brain and which extend almost to the residual lumen.

Although not shown in figure 9, the epithelial stalk is present. The infundibular process is still a simple ventro-posteriorly directed evagination from the floor of the third ventricle. In this stage the pars tuberalis reaches the floor of the brain. Later it encircles the infundibular stem.

Sixteen- and seventeen-day embryos. The 16-day gland (fig. 6) is more compact and approximates the proportions seen in the adult (figs. 8, 11). The caudal portion of the pars anterior is more pointed, however, and gives the gland a slipper-like appearance similar to that seen in the embryonic chick gland (Atwell '39). Not seen in this section (fig. 6) are the still prominent residual lumen, and the epithelial stalk, now reduced to one-fourth its original length, but still revealing a discontinuous lumen.

A cross section of the 17-day gland (fig. 7) shows the residual lumen, the lateral outpocketings of the infundibular process or neural lobe, and parts of the large carotid arteries. From a more anterior section of this same gland (fig. 10) can be seen the relation of the tuberalis to the pars anterior and

to the infundibulum. Both cross sections (figs. 7, 10) show the neural and epithelial lobes widely separated by connective tissue.

Later embryonic stages. During the following days the residual lumen and epithelial stalk are still present. On the twenty-third day the former disappears; the latter disappears about the twenty-fourth or twenty-fifth day. Thereafter a dense connective-tissue capsule develops around the gland.

The tuberalis has attained the proportions seen in the adult. In a great many of these later stages it may be as much as three and four cells in thickness in the region of the optic chiasma.

The adult gland. Except in size, the gland has changed very little since hatching. In many glands the tuberalis appears to be not as well developed as in the late embryonic stages. It tends to be discontinuous, made up of isolated groups or islands of cells. The infundibular process (pars nervosa or neural lobe) has the spearhead shape characteristic of all adult duck glands studied (figs. 8, 11). At the extreme posterior end of the pars anterior a part of the internal carotid artery can be seen (fig. 8). This is the point where the two internal carotids, which lie parallel to the lateral surfaces of the pars anterior, make an anastomosis. In the chicken the carotids lie in grooves lateral and perpendicular to the gland and make a ventral anastomosis (Atwell, '39). This gives the chick pituitary a bilobed appearance (Atwell, '39; Rahn, '39), a condition not seen in the duck. Of particular interest is the absence of a structural pars intermedia which is found in most other vertebrates.

II. Cellular differentiation in the pars buccalis

A. Cytology of the pars anterior of the sexually mature duck. The cells of the pars anterior are arranged in cords which are typically polygonal in cross section. Due to the frequent occurrence of lumina, which often contain colloid, the cords may present in section an acinous appearance. Lying between the cell cords are connective tissue and blood sinusoids.

Although the pars anterior of the duck pituitary does not give external evidence of a bilobed condition, it does reveal two cytologically different lobes. Atwell ('39) designated these lobes in the chick, "rostral" and "caudal," while Rahn ('39) employed the terms "cephalic" and "caudal." The latter terms were used by Rahn and Painter ('41) in the description of other avian pituitaries. In this more detailed account of the duck pituitary, the terms, "cephalic" and "caudal," refer to the anterior and posterior ends, respectively, of the pars anterior.

In the caudal lobe the predominating cells are the deep-red acidophiles which are concentrated largely in the dorsal two-thirds (fig. 8). In many glands, however, these cells are located more nearly in the center of the lobe and swing anteriorly and upward toward the tuberalis as depicted by deBeer ('26, plate VII, fig. 3). In such glands the periphery of the lobe is chiefly chromophobic, though the ventral zone may contain varying numbers of basophiles. Mixed with the acidophiles are both basophiles and chromophobes. The typical arrangement of the deep-red or "deep staining" acidophiles is on the periphery of the cell cord. However, they may bound or fill the cell cord exclusive of cells of other types. The granules, which are large, deeply stained, and usually packed closely together, are concentrated distally, toward the sinusoids. This results in the displacement of the nuclei toward the centers of the cords. The nucleoli, varying in number from one to five, stain bright red with azocarmine. If the differentiation is good, no other nuclear elements take or hold the stain. However, the nuclei in both acidophiles and basophiles are frequently pycnotic.

The acidophiles of the cephalic lobe are equally abundant and have the same position in the cell cords as the acidophiles of the caudal lobe. They differ, however, in their staining reaction, and in the possession of a finer granulation. After the Dawson fixation they stain yellow to light orange so that under low magnification they can be less readily recognized (fig. 8). After a Helly or Helly-osmic fixation, however, the

granules stain a reddish orange which renders the cells readily detectable under low magnification (fig. 11). Because of the somewhat smaller size of the cells and their tendency to lie almost exclusively on the periphery of the cell cord, they appear to have a more regular pattern than the acidophiles in the caudal lobe (fig. 11).

While the basophiles vary in number, size, and distribution in the adult gland, they are usually smaller and more numerous in the cephalic lobe. Particularly outstanding in number and size are the basophiles located in the posterior and dorsal region of the cephalic lobe, a region somewhat comparable to that designated in the cat and rabbit as the zona tuberalis (Dawson, '37). Like the acidophiles, the basophiles are frequently found in the center of the cell cord. Their usual position, however, is on the periphery. The nucleus may be either central or eccentric in position. If eccentric, it more often lies toward the proximal side of the cell. This leaves any secretion products concentrated on the distal side, next to the blood sinusoids. As in the acidophiles, the nucleoli have an affinity for azocarmine.

The character of the cytoplasm varies slightly with the fixative used. Aniline blue after Dawson's fluid usually reveals a distinct granulation. The mitochondria are well preserved, granular, and evenly distributed. After a Helly-osmic fixation the basophiles have an appearance like that of the basophiles of the chick, as described by Rahn ('39): "Their cytoplasm reveals either no or a very slight granulation which may be more adequately described as a flocculent precipitate." The affinity for aniline blue is greater and consequently the staining time must be less. The mitochondria are not as well preserved as when fixed with Dawson's fluid. Following either fixative, basophiles of varying shades of blue may be seen. Whether they should be classified as "dark staining" and "light staining" basophiles is questionable.

The basophiles of the male duck are of particular interest since a high percentage of them reveal vacuolation and frequently show hyalinization as well. Although this condition is

found in both young and old males, it is rarely, if ever, seen in the female.

As indicated above, the basophiles of the cephalic lobe are somewhat smaller and more numerous than those of the caudal lobe. However, the cytology and relations to the cells of other types are essentially the same for both.

The chromophobes in both lobes of the pars anterior are similar and are characterized by their small size, small amount of cytoplasm, indefinite cell border, and weak affinity for stain. Good differentiation leaves the nucleoli deep red and the cytoplasm light pink or colorless. These cells lie chiefly in the center of the cell cord though they frequently appear on the periphery. They are most numerous in the cephalic lobe. The ventral and posterior borders of the caudal lobe are often highly chromophobic.

B. The differentiation of cell types in the pars buccalis. From 9 through 12 days of incubation the cells of the pars anterior are undifferentiated. They have relatively large, clear nuclei, nucleoli which have an affinity for azocarmine, very little cytoplasm, and an indefinite cell boundary. The cells bordering the residual lumen bear a perpendicular relation to it, and may be called stratified columnar. Those further away have no particular orientation, while those on the periphery of the gland tend to be grouped in cords.

Little change takes place during the next 3 days. The nuclei of a few scattered cells of the cephalic lobe give a basophilic reaction. What this means is difficult to say since the nuclei of the earliest chromophiles are colorless.

At 16 days of incubation the first definite acidophiles and basophiles appear. They are about equal in number and are similar in size, in the possession of eccentrically located nuclei, and in the accumulation of distinct granules at the distal end of the cell. Of particular interest is the restriction of these cells to the highly vascularized cephalic lobe.

The basophiles are less numerous on the seventeenth day and by the nineteenth day are almost completely absent. The acidophiles show an increase, however, and a progressive ex-

tension toward the caudal lobe. By hatching time they are found fairly evenly distributed throughout the pars anterior.

During the first week after hatching the so-called "shift" of acidophiles to the caudal lobe becomes complete. Thereafter this lobe is characterized by the deep-staining acidophile. The light-staining acidophile takes its position in the cephalic lobe during the second week.

The basophiles have not been apparent since about the eighteenth day of incubation. By the end of the second week after hatching, however, they can be seen in the cephalic lobe. These post-natal basophiles are about the size and shape of the acidophiles, a condition noted in the 16-day-old embryo.

With the increase in age of the animal there is an increase in size, number and distribution of the basophiles. The data at hand seem to indicate that the basophiles of the sexually active animal are more numerous than those of the sexually inactive adult. Further observations are needed, however, before a more definite statement can be made.

The pars tuberalis of both the pre- and post-natal pituitary is made up of chromophobes similar to those of the pars anterior. Neither acidophiles nor basophiles, reported by Rahn ('39) in the tuberalis of the early chick pituitary, have been found.

DISCUSSION

As early as 33 hours of incubation Friedman ('34) found the hypophyseal area in the duck clearly defined. Lups ('29) observed that the anlage of Rathke's pocket appeared on the third day of incubation. Of some interest in connection with the early evagination of Rathke's pocket is the question of the part played by the endoderm. Although Pfeiffer ('25) reports that, in the chicken, Rathke's pocket alone goes into the make-up of the pars buccalis he found that Rathke's and Seessel's pockets contribute equally to its formation in the duck. Lups ('29) leaves open the possibility that endoderm may contribute to the formation of the caudal portion of the pars anterior. Friedman ('34), on the other hand, is of the opinion that there is no endodermal contribution.

Although the pars tuberalis was described by Economo (1899) and co-workers under different names, Tilney ('13) was the first to recognize and name it as a distinct structure. Atwell and Sitler ('18) report that in a 59-hour chick embryo, "two lateral enlargements of Rathke's pocket near its attachment to oral epithelium are exhibited. These are the anlagen of the lateral lobes from which the tuberal processes develop." According to Friedman ('34) the lateral lobes of the duck appear in the 96-hour embryo. The observations made in this work indicate that the tuberal processes do not reach the infundibulum until about the thirteenth day of incubation. They then proceed to extend dorsally and as far anteriorly as the optic chiasma and encircle the infundibular stem.

Lups ('29, fig. 10, p. 173) shows a continuous lumen in the hypophyseal stalk of the 7-day duck embryo. The writer finds the lumen discontinuous after 8 days, and the stalk persistent through the twenty-fifth day of incubation. Atwell ('39) reports the lumen discontinuous in a 7-day chick embryo, and the stalk persistent to the sixteenth day. In describing the morphology of the 9-day chick embryo Rahn ('39) states that the "hypophyseal stalk is no longer hollow," but does not say at what age the lumen disappeared.

The literature dealing with the question of the presence of an intermediate lobe in the avian pituitary is somewhat contradictory. Tilney ('13) pictures and describes as intermediate lobe or "pars infundibularis" in the chicken a small area of the caudal lobe lying next to the pars nervosa. He admits, however, that its cells pass imperceptibly into those of the pars anterior "without any distinct boundary line." Other investigators of the chicken pituitary, Haller (1898) and Herring ('08), interpret the tuberalis as pars intermedia. The same error is made by Gentes ('07) and Stendell ('14) for the duck, by Herring ('13) for the starling and thrush, and by Pokorny ('26) for six species of birds. Even Schooley and Riddle ('38), due no doubt to their misinterpretation of their sagittal sections, label the tuberalis "residual intermediate

lobe." DeBeer ('26), however, found no pars intermedia in either the fowl or duck.

How can the pars intermedia be recognized? Atwell ('39) presents the following criteria: "(1) Intimate relation with the neural lobe, and, (2) separation from the anterior lobe proper by the residual lumen." He reports that for a short time in the development of the chick these conditions are met. Rahn's ('39) findings are in complete agreement. The present investigation of the duck reveals similar facts. The residual lumen persists until shortly before hatching (figs. 1–4, 9). Certain early incubation stages show the caudal lobe closely applied to the ventral surface of the neural lobe. Later, however, connective tissue separates the two lobes and remains an integral feature in the adult gland. Except for occasional ciliated cysts whose cavities are believed to be remnants of the residual lumen, no sign of the lumen can be found in the gland of an animal following hatching. Hence, an important landmark is lost. Since there is no precise cytological distinction between the juxtaneural tissue and the rest of the tissue of the pars anterior, it can be said that there is no structural pars intermedia in the duck. The same statement can be made of the chicken (Rahn, '39), and sixteen other species of birds studied (Rahn and Painter, '41).

Of great interest is the appearance of the basophiles and acidophiles in the cephalic lobe of the 16-day old embryo. This position for the acidophiles is the reverse of that found in the gland after hatching. Rahn ('39) reports the appearance of the acidophiles in the cephalic lobe, and basophiles and chromophobes evenly distributed in the pars anterior of the chick of 10 and 11 days incubation. He finds the same reversal of the deep-staining acidophiles, and notes that fully differentiated basophiles do not develop until around 3 weeks after hatching. If the difference in the incubation periods, 21 days for the chick and 30 for the duck, be considered, there is seen a remarkable similarity in time of differentiation of these cellular elements.

It has been pointed out that the duck pituitary gives no external evidence of a bilobed condition, but does reveal two cytologically distinct zones designated the "cephalic" (anterior) and "caudal" (posterior) lobes. In the chicken (Rahn, '39) these two cytological zones coincide with the regions partially divided by the ventral and lateral grooves occupied by the internal carotids. The fact that the anastomosis of the carotids takes place at the extreme posterior end of the gland in the duck and most of the other birds described by Rahn and Painter ('41) probably explains the absence of a bilobed condition.

The chief elements that render the cephalic and caudal lobes distinct are the acidophiles. The deep-staining variety characterizes the caudal lobe (fig. 8), and extends anteriorly as far as an imaginary line drawn between the base of the tuberalis and the "former site of attachment of the hypophyseal stalk" (Rahn and Painter, '41). The light variety characterizes the cephalic lobe and after the Dawson fixation shows a fine yellow to yellowish-orange granulation. Although somewhat smaller, the basophiles of the cephalic lobe are more numerous than those of the caudal lobe.

SUMMARY

The morphogenesis, especially the cytogenesis, of the duck pituitary has been studied from 9 days of incubation to the adult condition.

1. At 9 days of incubation the infundibular process is a simple ventro-posteriorly directed evagination from the floor of the third ventricle. During progressively later stages it assumes the more complex, spearhead-condition found in the adult gland.

2. The pars tuberalis reaches the infundibulum about the thirteenth day of incubation. Its cells are chromophobic throughout all stages of development.

3. A structural pars intermedia never made its appearance.

4. Both the residual lumen and epithelial stalk disappear during the late embryonic stages, the former about the twenty-

third day, the latter about the twenty-fourth or twenty-fifth day.

5. At 16 days of incubation the first definite acidophiles and basophiles appear in the pars anterior.

a. Although the basophiles disappear in the late embryonic glands, they reappear in the cephalic lobe 2 weeks after hatching.

b. At the time of first appearance the acidophiles lie in the cephalic lobe. They gradually extend to the caudal lobe and by hatching time are evenly distributed throughout the pars anterior. These cells become the dark-staining acidophiles of the caudal lobe, while the light-staining variety appears in the cephalic lobe the second week after hatching.

6. Although not giving evidence of a bilobed condition, the pars anterior of the duck pituitary, due to the presence of two varieties of acidophiles, does reveal two cytologically distinct lobes.

ACKNOWLEDGMENT

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PLATES

ABBREVIATIONS

c.l., caudal lobe
c.c., cephalic lobe
e.s., epithelial stalk
i.c., internal carotid
i.p., infundibular process
m.c., mouth cavity
p.t., pars tuberalis
r.l., residual lumen
s.p., Seessel's pouch
v., third ventricle

Figure 3 is the only section shown whose anterior end points to left side of plate; all others point to right side.

PLATE 1

EXPLANATION OF FIGURES

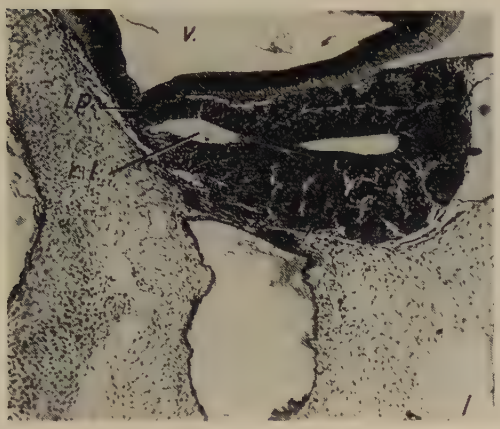
1 Parasagittal section of 10-day embryonic gland showing residual lumen and intimate relation between posterior end of gland and infundibular process. × 90.

2 Mid-sagittal section of 9-day embryonic gland showing relation between epithelial stalk and mouth cavity. × 84.

3 Mid-sagittal section through 11-day embryonic gland. × 96.

4 Mid-sagittal section of 12-day embryonic gland. × 99.

5 Mid-sagittal section of 10-day embryonic gland. × 88.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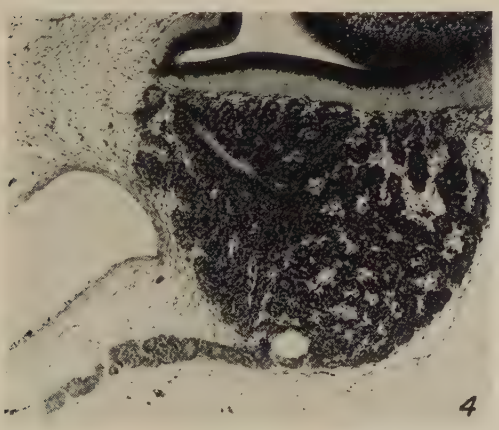
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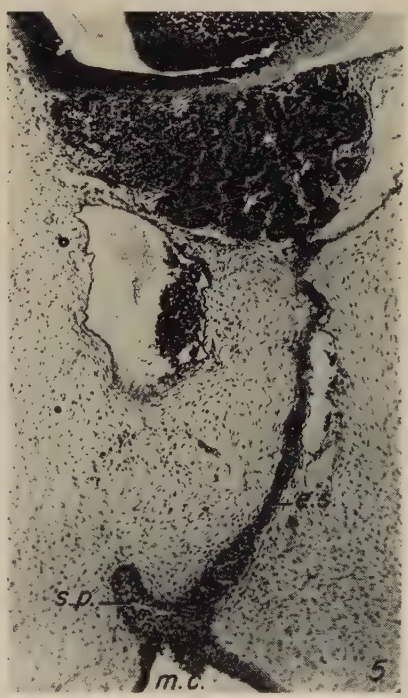
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PLATE 2

EXPLANATION OF FIGURES

6 Mid-sagittal section of 16-day embryonic gland. Notice more darkly stained anterior end in which is located the first acidophiles and basophiles. × 75.

7 Cross section of 17-day embryonic gland showing lateral outpocketings of infundibular process. × 68.

8 Mid-sagittal section of 5-month post-natal gland showing the dark-staining acidophiles restricted to the caudal lobe of pars anterior. Dawson's fixation. × 21.

9 Mid-sagittal section of 13-day embryonic gland. × 67.

10 Cross section of 17-day embryonic gland showing pars tuberalis. × 69.

11 Mid-sagittal section of 3-month post-natal gland showing the pattern-like distribution of the light-staining acidophile in the cephalic lobe. Helly fixation. × 19.

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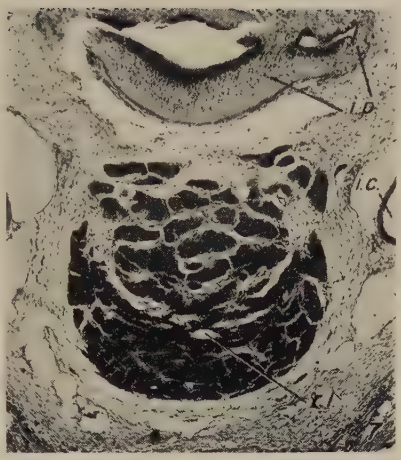
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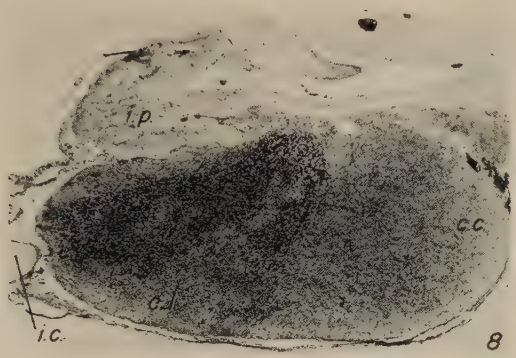
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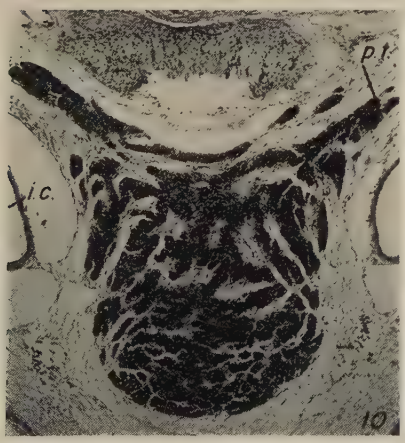
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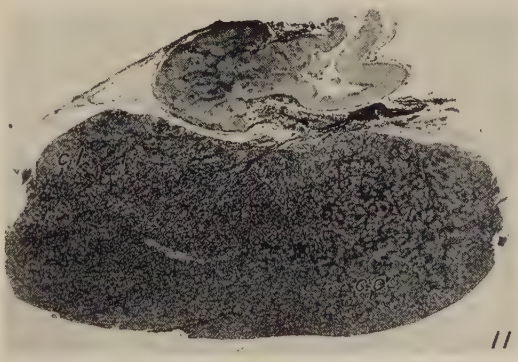
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EFFECTS OF LOCALIZED X-RADIATION ON REGENERATION OF THE URODELE LIMB

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FOUR FIGURES

INTRODUCTION

During recent years considerable attention has been given to an examination of the cellular changes which underlie regeneration of the amphibian limb. Reviews of the literature in this field have recently been presented by Butler and Puckett ('40), Schotté and Butler ('41), Butler and Schotté ('41) and Horn ('42). The central problem in a study of the cellular basis of regeneration concerns the source of the cells which enter into the establishment of the regeneration blastema. Using x-rays as a method of suppressing regenerative activity in the limb, Butler ('35) has demonstrated that the effectiveness of the radiation is due to its local action on cells in the limb and is not the result of its general action on the body of the animal as a whole. This conclusion is supported by the following evidence: (1) Radiation of a limb alone will prevent its regeneration. (2) An unradiated limb will retain the capacity for regeneration, although the remainder of the body of the animal has been radiated. (3) An unradiated limb transplanted to a radiated host will retain the capacity to regenerate. These observations are of particular significance, since they demonstrate that the cells which take part in regeneration arise in the limb, and are not derived from other regions of the body. These findings are in contradiction to earlier contentions of Hellmich ('29, '31), and others, that some of the cells which take part in regeneration of the

amphibian limb are of hematogenetic origin. Among the invertebrates, Puckett ('36), using Pennaria, and O'Brien ('42), using Nais, have also demonstrated the local nature of regenerative processes.

The present experiments were designed specifically to answer the question as to whether, by use of appropriate technique, regenerative capacity can be suppressed in a very restricted region of an organ, while closely adjacent regions continue to possess the capacity. Can the ability to regenerate be retained by the distal and proximal regions of an amphibian limb, while the mid-region of the limb is completely incapable of regenerating?

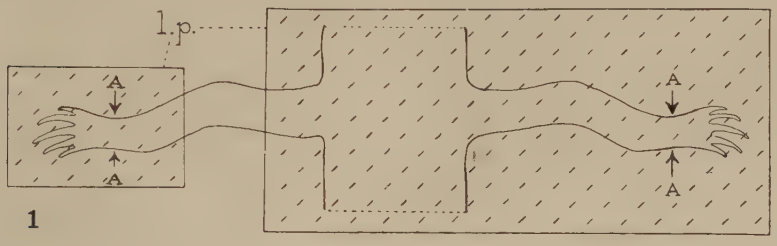
MATERIAL AND METHODS

The experimental material consisted of two groups of larvae of Eurycea bislineata, the individuals of one group measuring from 45–50 mm. in length, those of the other from 25–30 mm. Hind limbs only were used. In each case the left limb served as the experimental limb, the right as the normal control. At the time of radiation and amputation the larvae were narcotized in a 1:2000 solution of MS 222 (Schotté and Butler, '41). The source of unfiltered x-rays was a Coolidge medium focus tube operating at 60 KV and 6 ma. The larvae were placed 25 cm. from the target. Exposed portions of the limb were covered with 7 to 8 mm. of the narcotizing solution. For shielding portions of a larva, lead plates of at least 1.5 mm. thickness were used. As calibrated by a Victoreen r meter, the tube produced 85 r per minute. Dosages used were 2550 r, 2975 r and 5100 r.

EXPERIMENTAL

The initial procedure followed in the case of each animal is represented graphically in figure 1. The narcotized larva was completely shielded with lead, except a small portion of the left hind limb. The exposed portion included the knee joint and the region of the limb, about 1 mm. proximal and distal to

it. When placed under the x-ray tube, therefore, only this unshielded region of the left hind limb, measuring in all from 2 to 2.5 mm. in length, was subjected to the radiation.[1] Immediately after the radiation, both right and left hind limbs were amputated through the tarsals at the level indicated by arrows (A-A).



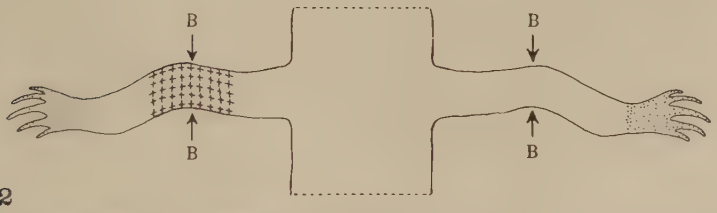


Fig. 1 Diagram of arrangement for localized radiation. The entire larva was shielded with lead plate (l.p.), with the exception of the region of the knee joint of the left hind leg. Arrows (A-A) indicate level of first limb amputation.

Fig. 2 Same larva 3 weeks after radiation and first limb amputation. Stippled areas represent regenerated structures; cross-hatching indicates region originally radiated; arrows (B-B) show level of second amputation.

The amputation through the unradiated tarsal region was followed by rapid and complete regeneration of right and left hind feet. These were completely formed within a period of approximately 3 weeks. The regenerated extremities are shown as lightly stippled areas in figure 2. Partial radiation of the limb, therefore, appears to have no effect on regenera-

[1] For the sake of simplicity, the diagrams show only the hind limbs and pelvic region of the body. Except for the designated region on the left hind limb, the entire animal was shielded with lead.

tion, when it is initiated in a shielded region distal to the radiated area.

At the end of 3 weeks a second amputation was made approximately at the level indicated by arrows (B-B) in figure 2. This involved the amputation of the left limb through the previously radiated region, now indicated by cross-hatching. The unradiated right leg was amputated at the same level.

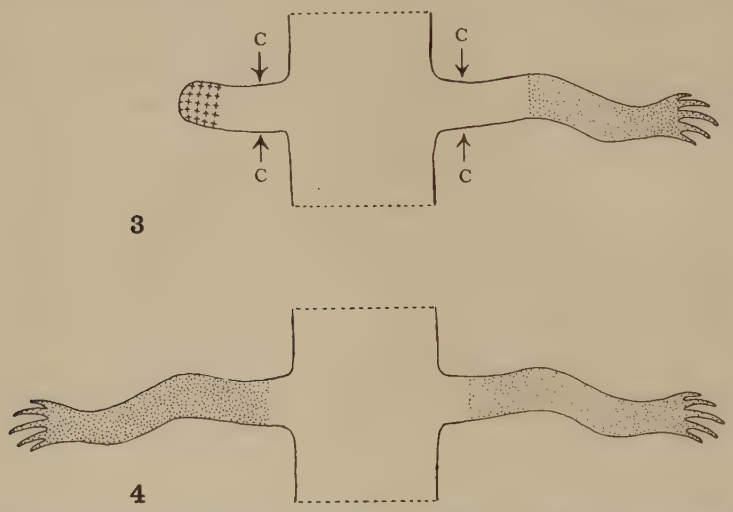


Fig. 3 Larva 9 weeks after second limb amputation. Stippled area represents regenerated structures; cross-hatching indicates non-regenerating radiated region; arrows (C-C) show level of third amputation.

Fig. 4 Same larva 4 weeks after third amputation. Stippled areas indicate regenerated limbs.

In some animals the amputation was through the distal end of the femur, in others directly through the knee joint, and in others through the proximal part of the tibia and fibula.

In cases where the dosage of 5100 r was used, results were clear-cut. The left limb, as shown in figure 3, failed completely to regenerate. The wound of amputation healed, but no regeneration blastema was established. The tip of the limb rounded over and became knob-like, and undoubtedly a certain amount of dedifferentiation occurred in the tissues of the radiated region (Butler, '33). Some larvae were observed for

as long as 9 weeks after the second amputation, without the slightest evidence of regeneration of the left limb. Local radiation, therefore, of a very restricted region is entirely effective in bringing about the loss of regenerative capacity in that region.

The unradiated right limb regenerated rapidly after the second amputation and ordinarily was completely formed in approximately 3 weeks.

Somewhat different results were obtained after the second amputation in those animals which received dosages of 2550 r and 2975 r. With a few exceptions, these dosages proved to be below the minimum effective dosage necessary for complete suppression of regeneration in Eurycea larvae of the ages used in these experiments. Consequently, in several cases incomplete regeneration took place; small imperfect feet were formed, lacking in some instances the full complement of digits. In other cases the response to the low dosages was expressed by greatly delayed, although eventually complete, regeneration. These observations on sub-minimal dosages are entirely in accord with the studies of Horn ('42) on the problem of x-ray dosage and inhibition of regeneration.

In order to determine the capacity for regeneration of the proximal portion of the limb, the mid-region of which was entirely incapable of regenerating, a third amputation was made at the level indicated by arrows (C-C) in figure 3. This amputation was performed from 7 to 9 weeks after the second amputation. The unradiated right limb served as a control. The results, as recorded 4 weeks later, are shown in figure 4. Starting from the unradiated proximal region of the left limb, a complete appendage regenerated. Thus, a limb, which for from 7 to 9 weeks had been incapable of manifesting its regenerative capacity, was enabled to express this capacity by a change in the level of amputation.

When these experiments were started, there were reasons to believe that, as a result of continued cellular dedifferentiation, progressing proximally from the level of amputation, the radiated area might eventually be resorbed and an active

level be established nearer the body, at which regeneration could be initiated. In the cases of total radiation, as carried out by Butler ('31, '33), and later workers, extreme dedifferentiation of the limb has been obtained in radiated animals, resulting in some cases in complete regression of the limb. Similar results, involving progressive dedifferentiation and regression in a proximal direction, have been obtained in denervated non-regenerating limbs by Schotté and Butler ('41) and Butler and Schotté ('41). In none of the present experiments, however, has there been a significant regression of the limb proximal to the level of amputation through the radiated region. The radiated region has no competence for initiating regeneration. The unradiated region exhibits its competence only after an amputation directly through it.

DISCUSSION AND SUMMARY

These experimental results provide clear-cut evidence that the regeneration process in certain essential features is strictly local in nature. The "building blocks" used in the establishment of the blastema, and eventually the new regenerate, have an exclusive and restricted origin at the site of amputation. Under the conditions of the foregoing experiments, the proximal portion of the thigh region can retain its full capacity to initiate regeneration of a complete limb, while a short distance away in the distal region of the thigh the capacity to regenerate is totally lacking. Likewise, when amputation is through the metatarsals, a new foot can be regenerated, although, had the amputation been made 2 or 3 mm. more proximally, no regeneration would have occurred. Whatever be the specific source or sources of the cells which enter into the formation of the regeneration blastema, their site of origin can no longer be in doubt. They arise at the level of amputation.

The body as a whole, through the mediation of nerves, and possibly other agents, exerts a definite and important influence on regeneration. Nevertheless, the materials used, and, to a considerable extent, the manner in which they are used, are the result of local activity.

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THE BLOOD PICTURE OF MATURE NORMAL DOGS

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As a result of experimental investigations designed specifically for the purpose, sufficient data to give a somewhat comprehensive evaluation of the blood cytology of the normal healthy canine is gradually accumulating. In this paper additional data, a compilation of some of the more recent observations and a comparison between the erythrocyte values of mature and immature canines will be presented.

The animals studied were sixty-six mature, mongrel dogs, forty-one males and twenty-five females, obtained in and around the vicinity of New York City. When the studies were made, the animals had become thoroughly acclimated to the cage conditions of the laboratory as well as to the diet which was adequate at all times. The animals were worm-free and in apparent good health when the blood samples were taken.

The techniques utilized to obtain the various erythrocyte values have been described in detail elsewhere (Landsberg, '39 and '40), and only the barest essentials will be given here. Blood (3.0 cc.) was obtained from the jugular vein by venipuncture using a dry needle and syringe. The blood was prevented from coagulating by the use of potassium and ammonium oxalate (2 mg. per cubic centimeter blood). This anticoagulant produces no change in the erythrocyte volume. The cells were counted by the usual procedure, the hemoglobin concentration was determined by the Newcomer colorimeter and the volume of packed red cells determined with a Wintrobe hematocrit. Each determination was carried out in duplicate and the mean taken as the representative value. The derived

corpuscular constants, i.e., mean corpuscular volume (c. v.), mean corpuscular hemoglobin (c. h.) and mean corpuscular hemoglobin concentration (c. c.) were calculated by the formula proposed by Wintrobe. The sedimentation rate was determined by the Wintrobe-Landsberg method, and the erythrocyte fragility was studied by the method given in Todd and Sanford ('39).

The statistical methods, employed for the analysis of the data, are those routinely used for the calculation of the standard deviation of the group and of the mean. As a test for significant difference, the difference between means was divided by the sigma of the difference; if this value was greater than three standard deviations, the difference was considered significant.

EXPERIMENTAL RESULTS

The mean erythrocyte count, hemoglobin concentration and volume of packed red cells are presented in table 1. Upon statistical analysis it was observed that there was no significant difference between the male and female groups, so the findings on the combined groups may be considered as the average value. For the combined male and female groups the mean erythrocyte count was 6.27 million per cubic millimeter, the hemoglobin 13.9 gm. per 100 cc. whole blood, and the volume of packed erythrocytes 44.3 cc. per 100 cc. of blood.

The values for the derived corpuscular constants are given in table 1 also. These values, similar to the determined values, did not show any statistical difference between the male and female groups. The mean corpuscular volume averaged 70.5 cubic microns, the mean corpuscular hemoglobin 21.8 micromicrograms and the mean corpuscular hemoglobin concentration 32.3% (table 2).

As the sedimentation rate for the individual male and female groups is not significantly different, the values for the combined group are given. The mean sedimentation rate, at the end of 1 hour, was 3.4 mm. with a standard deviation of 3.6 mm. for the group and a standard deviation of 0.50 mm.

TABLE 1

The mean and standard deviations for the erythrocyte values of forty-one male and twenty-five female, mature, normal dogs.

	MALE GROUP		FEMALE GROUP		COMBINED GROUPS	
	M ± S. D.	Sigma	M ± S. D.	Sigma	M ± S. D.	Sigma
Erythrocytes (millions per c.mm.)	6.28 ± .119	.756	6.15± .158	.792	6.27 ± .097	.788
Hemoglobin (grams per 100 cc.)	13.8 ± .351	2.18	14.1 ± .313	1.57	13.9 ± .233	1.88
Volume packed erythrocytes (cc. per 100 cc.)	44.4 ± .867	5.48	43.6 ± .692	3.46	44.2 ± .661	5.32
Mean corpuscular volume (cu.m.)	69.3 ± .840	5.31	71.3 ±1.08	5.40	70.55 ± .692	5.31
Mean corpuscular hemoglobin (micro-micrograms)	22.6 ± .487	3.08	21.2 ± .502	2.51	21.8 ± .347	2.80
Mean corpuscular hemoglobin conc. (per cent)	32.4 ± .522	3.30	32.2 ± .524	2.62	32.3 ± .384	3.10

M = mean. S. D. = sigma of mean. Sigma = standard deviation of group.

TABLE 2

Erythrocyte values of normal dogs as reported by various investigators.

INVESTIGATOR	NO. ANIMALS	R. B. C. × 10^6	Hb.	VOL. PK. R. B. C.	C.V.	C. H.	C. C.
			gm.	*cc.*	*cm.*	*vv.*	%
Dawson (5)	15	7.22	11.9	—	—	26.0	—
Musser and Krumbhaar (6)	47	5.97	15.5	—	—	—	—
Stucky and Rose (7)	12	—	15.0	—	—	—	—
Powers et al. (8)	25	7.00	15.8	45.2	64.5	23.6	35.0
Mayerson (9)	60	6.49	13.0	38.6	59.3	20.0	34.3
Leichsenring et al. (10)	32	7.17	14.1	47.7	66.5	19.7	29.6
Ashley and Guest (11)	50	6.87	16.0	45.6	66.6	23.6	39.4
Wintrobe et al. (4)	54	7.02	14.6	47.3	67.6	21.2	31.4
Morris et al. ('12)	31	6.20	15.1	—	—	24.3	—
Bruner and Wakerlin (13)	34	6.45	13.5	44.2	68.9	21.1	30.7
Crandall et al. (14)	25	6.20	12.9	—	71.3	20.9	—
Landsberg	66	6.27	13.9	44.2	70.5	21.8	32.3

for the mean. The erythrocyte fragility determinations are given also on the combined groups. Hemolysis began when the mean concentration of sodium chloride was 0.46% (sigma 0.004); hemolysis was complete when the mean saline concentration was 0.338% (sigma 0.007).

DISCUSSION

A comparison of the present data with that published for normal dogs by other investigators is given in table 2. This table with some modifications, however, is essentially a continuation of one published previously. In general, the present observations are in agreement with those published previously. It appears that the greatest discrepancies exist between the erythrocyte counts and hemoglobin concentrations published by different authors. Considered as a whole, the present results on normal dogs are in accord with the set statement of Morris ('40) that in the mature animal the erythrocyte values are influenced little by the age, breed or sex. Wells and Sutton ('15) pointed out that immature canines (puppies) have fewer erythrocytes than adult animals. This same observation has been reported by others. From his studies on a much larger series Morris ('40) again calls attention to this fact, and the present author, from studies on ninety-five immature dogs ('39), found the erythrocyte values to be lower. Since the present study on mature animals and the previous investigations on immature dogs (Landsberg, '39) had been carried out by the same techniques and the same investigator, it seemed a comparison between them would be valid.

Such a comparison has been made and is presented in table 3. The difference between the values obtained for the mature and immature dogs when tested statistically was significantly different; this difference applied equally to the determined and derived erythrocytic observations. The determined erythrocyte values, namely, the red cell count, the hemoglobin and the hematocrit are lower in the immature animals than in the adults. Of the derived erythrocytic con-

stants the mean corpuscular volume is significantly larger in the immature dogs indicating a definite increase in the size of the red corpuscle; the mean corpuscular hemoglobin and hemoglobin concentration is lower in the young animals.

There is also another difference between the immature and adult groups which merits attention. In a previous publication (Landsberg, '39) it was pointed out that immature female

TABLE 3

Comparison of the measured and derived erythrocyte constants of normal, immature and mature canines.

	IMMATURE DOGS		MATURE DOGS		DIFFERENCE
	Mean	S. D.	Mean	S. D.	Sigma difference
Erythrocytes[1]	5.00	.068	6.27	.097	18.6 sigma
Hemoglobin	8.9	0.15	13.9	0.23	14.8 sigma
Hematocrit	37.8	0.46	44.2	0.66	8.0 sigma
Mean corp. vol.	76.3	0.56	70.6	0.69	6.4 sigma
Mean corp. Hb.	18.6	0.25	21.8	0.35	7.4 sigma
Mean corp. Hb. conc.	24.4	0.33	32.3	0.38	15.8 sigma
Number of animals	95		66		

[1] Units the same as in tables 1 and 2.

S. D. = standard deviation of mean.

canines had a significantly higher erythrocyte count, hemoglobin concentration and volume of packed red cells than the males. This difference, furthermore, could not be explained on the basis of an age differential between the two sexes. The mature animals did not show such a difference between the male and female groups (table 1).

SUMMARY

1. Some data on the blood picture of sixty-six normal, adult, male and female canines are presented. The present data are compared with previous observations on animals in a similar category. A comparison between the erythrocyte value of immature and adult dogs is given.

2. The mean erythrocyte count was 6.27 million per cubic millimeter; the hemoglobin concentration 13.9 gm. per 100 cc.

whole blood; the volume of packed red cells 44.2 cc. per 100 cc. blood.

3. The mean corpuscular volume averaged 70.5 cubic microns; the mean corpuscular hemoglobin 21.8 micro-micrograms; and the mean corpuscular hemoglobin concentration 32.3%.

4. The mean sedimentation rate at the end of 1 hour was 3.4 mm.

5. Observations on erythrocyte fragility showed hemolysis began when the saline concentration was 0.46% and was complete at 0.33%.

6. Statistically there is no significant difference in the hematological values for adult male and female canines.

7. Statistically there is a significaant difference in the blood picture of adult animals when compared with immature animals.

The present observations on the sedimentation rate are in accord with those recently published (Simms, '40). Using the Wintrobe sedimentation tube, this author found that eighteen blood samples from fifteen apparently normal dogs ranged from 1 to 4 mm. The mean sedimentation rate for the present series is 3.4 mm. at the end of 1 hour.

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BOOK REVIEW

VISUAL MECHANISMS. Edited by Heinrich Klüver. The Jacques Cattell Press, Lancaster, Pa. 8vo. 8 + 322 pp. 1942.

This volume is an outgrowth of one of the symposia dealing with "New Frontiers in Education and Research" held in celebration of the Fiftieth Anniversary of the University of Chicago in 1941. The eight papers presented there have since undergone expansion, and four additional contributions have been intercalated. Even so, it is not pretended that all aspects of the subject "Visual Mechanisms" are covered; especially is this true since current emergencies have made it impossible for some of the prospective additional collaborators to furnish manuscripts. Rather it is held that the chief value of the present volume lies "in providing a clear picture of the diverse methods and points of view found in modern studies of vision." In its published form this collection of original contributions and correlating reviews appears as volume 7 in the series of "Biological Symposia" edited by Jacques Cattell.

The diversity of the topics discussed and the caliber of the contributors can be seen from the following listing:

Energy relations in vision. Selig Hecht.

The photochemistry of visual purple. A. C. Krause.

Visual systems and the vitamins A. George Wald.

Anoxia in relation to the visual system. E. Gellhorn.

Visual sensation and its dependence on the neurophysiology of the optic pathway. S. H. Bartley.

Alpha waves in relation to structures involved in vision. T. J. Case.

Recent evidence for neural mechanisms in vision leading to a general theory of sensory activity. W. H. Marshall and S. A. Talbot.

The functional organization of the occipital lobe. G. v. Bonin, H. W. Garol and W. S. McCulloch.

Anatomy of the retina. S. L. Polyak.

The visual cells and their history. G. L. Walls.

Functional significance of the geniculo-striate system. H. Klüver.

The problems of cerebral organization in vision. K. S. Lashley.

These are not stereotyped topics or treatments. They really deal with "New Frontiers." It is scarcely practicable to attempt to

summarize in a review even the more striking findings recorded and the conclusions reached. Moreover, since the topics treated are selective and do not integrate into a fully rounded whole, no two reviewers could be expected to agree on what sample wares are most deserving of such display. It is probably the wisest course to let the bare list of topics and names of their sponsors tempt the interest of those who seek the latest information in new ramifications of an old field. Relatively few readers will perhaps be interested in all the topics presented. Probably few will thoroughly understand or be able to appraise them all. This is foreseen in the Foreword wherein it is mentioned that "it was told of Charles Darwin by his son that he got a kind of satisfaction in reading articles which (according to himself) he could not understand."

L. B. Arey,
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REVIEWS OF APPROVED FILMS IN ANATOMY AND BIOLOGY

The following films in biology have been approved by one or more members of the Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute Committee.

Committee of the American Association of Anatomists:
Eliot R. Clark, Warren H. Lewis, Carl C. Speidel, and consultants.

Committee of the American Society of Zoologists:
Ralph Buchsbaum, Robert Chambers, Oscar W. Richards, and consultants.

Committee of The Wistar Institute of Anatomy and Biology:
Edmond J. Farris, Cranford Hutchinson, John S. Nicholas, and others as appointed.

TROPICAL OPOSSUMS

EDMOND J. FARRIS

(400 feet, kodachrome, silent)

In this reel Dr. Farris presents 3 notable additions to laboratory animals available for embryological research, for he shows pictures of 3 genera of small American marsupials captured in Panama and brought to the E. B. Morris Biological Farm of The Wistar Institute of Anatomy and Biology, where the animals are being bred and reared, and where the pictures were taken. The types shown in the film are the Metachirus ("woolly"), the Metachirops ("gray masked") and the Marmosa isthmica (murine). They range in size from that of a mouse to that of a large-sized laboratory rat. The film includes views of offspring, raised from pouch young, after one year in captivity, showing, incidentally, the feasibility of domestication; and of pouch young of various developmental ages. If these small marsupials can be raised and maintained in quantity in laboratories, Dr. Farris will have made a contribution of very great importance. It is difficult to imagine any person interested in experimental embryology who, on seeing this film, would not wish to start a colony of one or more of the 3 species in his laboratory. Furthermore, zoologists should find the film useful in teaching.

Photography is excellent. Composition, length of legends and of scenes are up to professional standards. ★A — E. R. Clark.

A RAPID PREGNANCY TEST — 2 to 6 HOURS

U. J. SALMON, S. H. GEIST AND C. S. POOLE

(250 feet, kodachrome, silent)

This short reel shows the technique for performing a two to six hour test for human pregnancy. Immature rats (22–25 days) were injected subcutaneously with 2 c.c. of night specimen of urine, and

autopsied at the end of two, four, and six hours. The chorionic gonadotropin caused a marked hyperemia of the ovaries with a red color change, in 94 per cent in two hours, and in 98 per cent in six hours. Sections of ovaries six hours after injection with pregnancy urine showed a perifollicular vascular dilatation that was lacking in the controls.

Photography is good, but the titles are unusually long.
B — Edmond J. Farris.

MATERNAL BEHAVIOR IN THE RAT AND ITS MODIFICATION BY CORTICAL INJURY

FRANK A. BEACH

(350 feet, monochrome, silent)

In the beginning the film shows normal nest building, retrieving and defense behavior of a black rat. An operate female of the same species with one-third of her cortex removed is introduced into the same kind of cage situation. Lack of integration of behavior is shown in that she builds two poor nests instead of one. In contrast, also, with the normal female the operate animal retrieves her young incoördinately. The normal female removes her nest and litter to a sheltered corner of the cage when an air blast is turned on the nest and young. In the same situation the operate animal made an abortive attempt to remove her young but did not complete the removal. Instead she built a nest for herself in another part of the cage. The film shows clearly the non-adaptiveness of the behavior of the operate rat as compared with that of the normal intact animal.
★B — C. R. Carpenter.

PRACTICAL PREVENTIVE ORTHODONTICS

M. J. FUTTERMAN — WITH ASSISTANCE OF LEO SCHWARTZ

(800 feet, kodachrome, silent)

This film is designed as a teaching film for dental students. First are shown several types of tooth abnormalities including a supernumerary lower incisor, prolonged retention of a deciduous tooth, and lingual displacement of incisors; and some of their harmful effects are pointed out. (This portion would be improved by a sound track.)

The major part of the film is taken up with the technic of correction. Selecting a simple case of lingual displacement of an incisor, the author gives a complete picture of the construction of the corrective appliance from the first mould to the finished and installed apparatus. Finally the replacement of prematurely lost deciduous teeth is briefly pictured.

The color photography is excellent. The story is well told and the film should be of value in the teaching of orthodontic technic to dental students. B — Eliot R. Clark

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★ Copy deposited in the Film Depository of The Wistar Institute, and is available to all scientists for use at The Wistar Institute.

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Meeting Postponed by Request of Office of Defense Transportation

PROGRAM OF THE FORTIETH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOOLOGISTS

NEW YORK CITY

DECEMBER 29, 30, 31, 1942

The Society this year will meet in conjunction with Section F of the American Association for the Advancement of Science and in association with several other biological societies. All Meetings for the presentation of papers, symposia, and demonstrations will be held in the halls of Hunter College, 695 Park Avenue, New York City.

As in recent years several special sessions have been arranged, all of which will be held jointly with other societies. A symposium on "Insects and the War" organized by Dr. Roger B. Friend will be given in two sessions, and sponsored jointly with the American Association of Economic Entomologists, The Ecological Society of America, and The Entomological Society of America. A symposium on "Immunology as a Tool in Biological Research" organized by Dr. Malcom R. Irwin will be held jointly with the Genetics Society of America. In addition, the Society will join with the American Society of Naturalists in sponsoring a symposium on "The Naturalist in America."

The Biologists' Smoker will be held on the first evening of the meeting, Tuesday, December 29th, at 9:00 o'clock, in the West Ball Room of the Commodore Hotel. Admission will be by A.A.A.S. registration badge or receipt. There will be a registration desk at the Commodore Hotel on the night of the Smoker for those who have not had a previous opportunity to register.

The annual dinner of the American Society of Zoologists, to which all zoologists and friends are invited, will be held

at 7:00 o'clock Wednesday evening, December 30th, in the East Ball Room of the Commodore Hotel. Dr. Warder Clyde Allee, Vice-President of Section F of the American Association for the Advancement of Science, will deliver the annual address on "Where Angels Fear to Tread."

The Secretary has received no notification of special reduction in railroad fares for the New York meeting. However, members are advised to consult their local railroad agents in regard to possible convention or holiday rates. All those planning to attend the meeting are urged to make reservations early.

As in former years the Society is greatly indebted to The Wistar Institute for prompt publication and mailing of this program.

LOCATION OF MEETINGS

Hunter College of the City of New York, in which all sessions will be held, is located in a large, new, spacious building on Park Avenue between 68th and 69th Streets, and may be reached rapidly by bus or subway from the Commodore Hotel at 42nd Street. A northbound bus may be taken at Lexington Avenue and 42nd Street, or any uptown *local* subway at the station in the hotel. Both stop at 68th Street. Walk to 69th Street and turn toward Park Avenue. Those preferring to walk can go directly up Lexington Avenue or go east on 42nd to Fifth Avenue and walk up Fifth Avenue to 69th Street and across to Park Avenue. *Only the 69th Street entrance of the College will be open.*

DINNER TICKETS

Tickets for the Zoologists' Dinner on Wednesday evening will be on sale at the Information Desk in Hunter College. In order to assist the local committee in making arrangements, all persons planning to attend the Dinner are urged to purchase tickets as early as possible.

LUNCHEONS

No arrangements for special Biologists' Luncheons have been made this year. However, arrangements have been made for adequate luncheon facilities in the Hunter College cafeteria for the 3 days of the meeting. The neighborhood includes a few small tea rooms, whose location, prices, and capacity will be listed at the Information Desk.

The North and South lounges on the third floor of Hunter College will be open all day on the 29th, 30th, and 31st, and tea will be served in the afternoons.

PROGRAM

The Retiring President of the American Association for the Advancement of Science, Dr. Irving Langmuir, General Electric Company, Schenectady, New York, will deliver his address in the Grand Ball Room of the Pennsylvania Hotel at 8:30 P.M. on Monday, December 28th. Immediately following the address, the local committee will tender a reception to officers and members of the Association and their guests.

TUESDAY, DECEMBER 29TH

9:30 A.M. Section A. *Endocrinology.* Papers 1 to 11. Room 619, Hunter College.
Section B. *General Physiology.* Papers 12 to 20. Room 620, Hunter College.
Section C. *Embryology.* Papers 21 to 33. Room 718, Hunter College.

2:00 P.M. Section A. Joint session with the Genetics Society of America, Assembly Hall, Hunter College.
Symposium on *"Immunology as a Tool in Biological Research,"* Dr. E. W. Lindstrom, presiding.

1. Immunochemical approaches to biological problems. Michael Heidelberger, Columbia University.
2. Evolution of the human blood group factors. Alexander S. Wiener, Office of Chief Medical Examiner of New York City.
3. Immunogenetic studies of species relationships. Malcom R. Irwin and Russel W. Cumley, The University of Wisconsin.
4. Serology and animal systematics. Alan Boyden, Rutgers University.

2:30 P.M. Section B. Joint session with the American Association of Economic Entomologists, The Ecological Society of America, and The Entomological Society of America.
Symposium on *"Insects and the War,"* Dr. W. H. White, presiding. Program on page 439. Grand Ball Room, Hotel New Yorker.

9:00 P.M. Biologists' Smoker. West Ball Room of the Commodore Hotel.

WEDNESDAY, DECEMBER 30TH

9:00 A.M. Section A. Joint session with the American Association of Economic Entomologists, The Ecological Society of America, and The Entomological Society of America.
Symposium on *"Insects and the War"* (concluded). Dr. Lorande L. Woodruff, presiding. Program on page 440. Assembly Hall, Hunter College.
Section B. *General Physiology.* Papers 48 to 60. Room 619, Hunter College.
Section C. *Cytology and Protozoology.* Papers 61 to 71. Room 620, Hunter College.

12:00 M. Annual Business Meeting of Section F, A.A.A.S., Room 619, Hunter College.

12:10 P.M. Annual Business Meeting of the American Society of Zoologists, Room 619, Hunter College.

2:00 P.M. *Demonstration Program.*[1] Papers 105 to 121. Room 720, Hunter College. (Motion pictures in rooms 724 and 722, Hunter College.) See page 12.

7:00 P.M. *Zoologists' Dinner.* Address by Dr. Warder Clyde Allee, Vice-President of Section F, A.A.A.S., on "Where Angels Fear to Tread." East Ball Room of the Commodore Hotel.

THURSDAY, DECEMBER 31ST

9:30 A.M. Section A. *Endocrinology.* Papers 72–82. Room 619, Hunter College.
Section B. *Cellular Physiology.* Papers 83–91. Room 620, Hunter College.
Section C. *Parasitology, Morphology, and Ecology.* Papers 92–100. Room 718, Hunter College.

2:00 P.M. Joint session with the American Society of Naturalists, Assembly Hall, Hunter College.
Symposium on "The Naturalist in America," Dr. Ralph E. Cleland, President of the American Society of Naturalists, presiding.

1. The anniversary of the American naturalist. L. C. Dunn, Columbia University.
2. The nature of fluctuations. Selig Hecht, Columbia University.
3. The future of the naturalist in America. Paul B. Sears, Oberlin College.
4. (Title to be announced.) Enrique Beltran, Department of Public Health, Mexico City.

The Annual Dinner of the American Society of Naturalists will be held on Thursday evening, December 31st, at 6:30 P.M. in the Rose Room of the Commodore Hotel. Dr. E. G. Conklin, Princeton University, will make some remarks in honor of "The American Naturalist." The presidential address will be given by Ralph E. Cleland, Indiana University, on the subject "The Problem of Species in Oenothera."

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[1] Attention is also directed to the demonstration program of the Genetics Society of America on Wednesday afternoon in rooms 812 and 815, Hunter College.

LIST OF TITLES

TUESDAY MORNING SESSION, DECEMBER 29TH

Hunter College, New York City

This session will be held in three sections, as shown below.

Section A. *Endocrinology*, 9:30 A.M.
Room 619, Hunter College

1. Relative sensitivity to certain ovulation-inducing agents of first and subsequent follicles of clutch sequences in the hen. (12 min.) (Lantern.) R. M. Fraps and A. Dury, U. S. D. A., Beltsville, Md.
2. Studies on the reproduction of viviparous snakes. (15 min.) (Lantern.) Douglas E. Bragdon, Harvard University.
3. Effects of a neurophysiological stimulus on the breeding of albino rats. (15 min.) (Lantern.) Edmond J. Farris and Eleanor H. Yeakel, The Wistar Institute of Anatomy and Biology.
4. Studies of reproduction in the albino rat. (15 min.) (Lantern.) Edmond J. Farris, The Wistar Institute of Anatomy and Biology.
5. The influence of age, season and pregnancy on the character of the reproductive cycle in 22 chimpanzees. (15 min.) (Lantern.) William C. Young and Robert M. Yerkes, Yerkes Laboratory of Primate Biology.
6. Quantitative studies on hormonal sex reversal in larval anurans. (15 min.) (Lantern.) Emil Witschi, Charles Foote and Beatrice Mintz, State University of Iowa.
7. Deposition of melanin in the sparrow's bill as a response specific to androgens. (15 min.) (Lantern.) Carroll A. Pfeiffer, Charles W. Hooker and Arthur Kirschbaum, Yale University.
8. The preparation and biological assay of relaxin. (15 min.) (Lantern.) A. A. Abramowitz, F. L. Hisaw, L. H. Kleinholz, W. L. Money, R. V. N. Talmage and M. X. Zarrow, Harvard University.
9. Relation of progesterone to the formation of relaxin. (15 min.) (Lantern.) F. L. Hisaw, M. X. Zarrow, R. V. N. Talmage, W. L. Money and A. A. Abramowitz, Harvard University.
10. Effects of estrogen and testosterone on mammary glands of hypophysectomized rats. (15 min.) (Lantern.) Samuel L. Leonard, Cornell University.
11. The structure, function and seasonal changes of the so-called "airsacs" of the sage grouse. (15 min.) (Lantern and motion picture projector.) Hermann Rahn, Floyd L. Clarke and James R. Simon, University of Rochester and the University and Game and Fish Commission of Wyoming.

TUESDAY MORNING SESSION, DECEMBER 29TH

Section B. *General Physiology*, 9:30 A.M.

Room 620, Hunter College

12. On the genetic basis for hermaphroditism. (15 min.) (Lantern.) John W. Gowen, Iowa State College.
13. The effect of temperature on the expression of intersexuality in Daphnia longispina. (15 min.) (Lantern.) Katherine Sanford, Allegheny College. (Introduced by A. M. Banta.)
14. Relative growth gradients and transformed coordinates related in the analysis of growth. (12 min.) (Lantern.) Oscar W. Richards and Arthur J. Kavanagh. Research Department, Spencer Lens Co., Buffalo.
15. The normal changes in weight and water content during the life cycle of the wood-eating beetle, Passalus cornutus Fab. (15 min.) (Lantern.) I. E. Gray, Duke University.
16. Protyrosinase and a liquid-air interface. (15 min.) (Lantern.) T. H. Allen, A. B. Otis and J. H. Bodine, State University of Iowa.
17. Amylase activity of human parotid and submaxillary-sublingual saliva. (15 min.) (Lantern.) E. Alfred Wolf and John D. O'Neal, University of Pittsburgh.
18. The effect of exogenous adrenalin on frog renal haemodynamics. (15 min.) (Lantern.) Roy Philip Forster, Dartmouth College.
19. Responses of the capillary bed in the frog to adrenal cortical hormones. (12 min.) (Lantern.) B. W. Zweifach and Robert Chambers, Department of Biology, Washington Square College, New York.
20. The effect of Kendall's cortical extracts on kidney tubules in tissue culture. (15 min.) (Lantern.) Robert Chambers and Gladys Cameron, Department of Biology, Washington Square College, New York.

TUESDAY MORNING SESSION, DECEMBER 29TH

Section C. *Embryology*, 9:30 A.M.

Room 718, Hunter College

21. Hormones in Drosophila. (15 min.) (Lantern.) Dietrich Bodenstein, Columbia University.
22. The early embryonic development of Amaroecium constellatum. Sister Florence Marie Scott, Seton Hill College.
23. Studies on the development of the kidney of the eel. (10 min.) (Lantern.) Raymond Haringa and Charles G. Dobrovolny, Biological Institute of New Hampshire.
24. The formation of the blastodisc in the egg of the zebra fish, Brachydanio rerio. (15 min.) (Lantern, motion picture projector.) Warren H. Lewis, The Wistar Institute, and Edward C. Roosen-Runge, Brown University.
25. Effects of different degrees of polyploidy on viability, growth, and metamorphosis of larvae of the newt, Triturus viridescens. (15 min.) (Lantern.) Gerhard Fankhauser and Rita C. Watson, Princeton University.

26. The origin of subcutaneous melanophores. (5 min.) (Lantern.) Hans Elias, Elizabeth Quigg and Edward Sauris, Middlesex University.
27. Sex reversal and the genetics of sex determination in axolotl (Amblystoma mexicanum). (15 min.) (Lantern.) R. R. Humphrey, Department of Anatomy, University of Buffalo.
28. Growth of the frog kidney carcinoma when transplanted to the tails of normal and permanent tadpoles. (15 min.) (Lantern.) Robert Briggs and Ronald Grant, Lankenau Hospital Research Institute and McGill University.
29. Stimulation of the hen's egg by ultraviolet. (10 min.) (Lantern.) Alexis L. Romanoff, Cornell University.
30. The development and regression of the primitive streak in the explanted blastoderm marked with carbon particles. (15 min.) (Lantern.) Nelson T. Spratt, Jr., The Johns Hopkins University.
31. Effects of localized x-radiation on regeneration of the urodele limb. (10 min.) (Lantern.) E. G. Butler and J. P. O'Brien, Princeton University and De Paul University.
32. The effect on the embryos of x-radiation of the male rat. (15 min.) (Lantern.) Margaret Henson, New York University. (Introduced by Roberts Rugh.)
33. Rib ossification patterns in 12- and 13-ribbed rabbits (15 min.) (Lantern.) Inez Baumgartner, Brown University.

TUESDAY AFTERNOON SESSION, DECEMBER 29TH

This session will be held in two sections, as shown below.

Section A. *Symposium*, 2:00 P.M.

Joint Session with the Genetics Society of America

"IMMUNOLOGY AS A TOOL IN BIOLOGICAL RESEARCH"

Dr. E. W. Lindstrom, presiding

1. Immunochemical approaches to biological problems. Michael Heidelberger, Columbia University.
2. Evolution of the human blood group factors. Alexander S. Wiener, Office of Chief Medical Examiner of New York City.
3. Immunogenetic studies of species relationships. Malcom R. Irwin and Russel W. Cumley, The University of Wisconsin.
4. Serology and animal systematics. Alan Boyden, Rutgers University.

TUESDAY AFTERNOON SESSION, DECEMBER 29TH

Section B. *Symposium*, 2:30 P.M.

Grand Ball Room, Hotel New Yorker

Joint session with the American Association of Economic Entomologists, The Ecological Society of America, and The Entomological Society of America.

"INSECTS AND THE WAR"

Dr. W. H. White, presiding

1. Entomological problems imposed by war conditions. P. N. Annand, U. S. Bureau of Entomology and Plant Quarantine, Washington, D. C.
2. (a) Entomology and malaria control in war areas. L. L. Williams, Jr., U. S. Public Health Service, Washington, D. C.
   (b) Some entomological aspects of troop mobilization. Lt.-Col. W. V. King, Bureau of Entomology, Orlando, Florida.

3. Entomology and food mobilization. W. P. Flint, State Department of Entomology, Urbana, Illinois.
4. Agricultural insecticides in relation to critical war materials. R. C. Roark, U. S. Bureau of Entomology and Plant Quarantine.
5. Taxonomy and the war. C. F. W. Muesebeck, U. S. Bureau of Entomology and Plant Quarantine.
6. Training entomologists for service in the armed forces. C. L. Metcalf. University of Illinois.

TUESDAY EVENING SESSION, DECEMBER 29TH

Biologists' Smoker, 9:30 P.M.; West Ball Room of the Commodore Hotel

WEDNESDAY MORNING SESSION, DECEMBER 30TH

This session will be held in three sections, as shown below.

Section A. *Symposium*, 9:30 A.M.

Assembly Hall, Hunter College

Joint session with the American Association of Economic Entomologists, The Ecological Society of America, and The Entomological Society of America.

"INSECTS AND THE WAR"

Dr. Lorande L. Woodruff, presiding

1. The insect problem. R. B. Friend, Connecticut Agricultural Experiment Station and Yale University.
2. Insects and food production. P. N. Annand, U. S. Bureau of Entomology and Plant Quarantine, Washington, D. C.
3. The health of man and animals as affected by insects. F. C. Bishopp, U. S. Bureau of Entomology and Plant Quarantine, Washington, D. C.
4. The efficient use of insecticides. F. L. Campbell, Ohio State University.

WEDNESDAY MORNING SESSION, DECEMBER 30TH

Section B. *General Physiology*, 9:30 A.M.

Room 619, Hunter College

48. Acidosis in the rabbit during "gravity shock." (15 min.) (Lantern.) William H. Cole, James B. Allison, James H. Leathem, William L. Nastuk and John A. Anderson, Rutgers University.
49. Alteration of frequency of pacemaker nerve cells by imposed direct current. (15 min.) (Lantern.) T. H. Bullock, Yale University.
50. Effect of direct and diffuse light on the copepod Acartia tonsa. (15 min.) (Lantern.) William Schallek, Harvard University.
51. Spacial relations of ommatidia in insects and differential sensitivity to moving visual stimuli. (12 min.) (Lantern.) Ernst Wolf, Harvard University.
52. Reestablishment of visuomotor coordinations by optic nerve regeneration. (15 min.) (Lantern.) R. W. Sperry, Harvard Biological Laboratories. (Introduced by A. B. Dawson.)

53. The effects of muscle length and shock intensity on muscular latency-relaxation. (5 min.) (Lantern.) Alexander Sandow, Washington Square College of Arts and Science.
54. The effect of tetanus on muscular latency-relaxation in normal and iodo-acetate-poisoned muscles. (5 min.) (Lantern.) Alexander Sandow, Washington Square College of Arts and Science.
55. On the significance of muscular latency-relaxation. (5 min.) (Lantern.) Alexander Sandow, Washington Square College of Arts and Science.
56. The effects of insulin on the responses of the frog's heart and rectus abdominis to acetylcholine. (15 min.) (Lantern.) John H. Welsh, Harvard University.
57. The structure, development, and discharge of the penetrant of Pelmatohydra oligactis (Pall). (15 min.) (Lantern.) William A. Kepner, Bruce D. Reynolds, Lewis Goldstein and Herbert Taylor, Miller School of Biology, University of Virginia.
58. The influence of $CaCl_2$, NaCl and osmotic pressure on the size of food vacuoles in Vorticella. (10 min.) (Lantern.) William J. Bowen, University of North Carolina.
59. Concerning the directness of effect of factors which influence the size of food vacuoles in the ciliate Vorticella. (5 min.) (Lantern.) William J. Bowen, University of North Carolina.
60. Electron microscope studies of the structure of paramecium trichocysts. (15 min.) (Lantern.) Marie A. Jakus, Cecil E. Hall and Francis O. Schmitt, Department of Biology and Biological Engineering, Massachusetts Institute of Technology.

WEDNESDAY MORNING SESSION, DECEMBER 30TH

Section C. *Cytology and Protozoology*, 9:30 A.M.

Room 620, Hunter College

61. A possible source of new evidence on the difference between mitosis and meiosis. (15 min.) (Lantern.) C. A. Berger and Sr. Mary Grell, Fordham University.
62. Some effects of centrifugal force upon the polarity of germinating pollen grains. (15 min.) (Lantern.) H. W. Beams and Norman Sulkin, State University of Iowa.
63. The chromatophores of Rana pipiens. (15 min.) (Opaque projector.) Hans Elias, David Cohen and Irving Lieberman, Middlesex University.
64. Nuclear phenomena in the mouse liver from birth to maturity. (8 min.) (Lantern.) J. Walter Wilson, Brown University.
65. The cytology of the mouse liver in a controlled diurnal cycle. (7 min.) (Lantern.) Helen Wendler Deane, Brown University. (Introduced by J. Walter Wilson.)
66. Destructive changes in the pancreas in old age, shown by a study of Wistar Institute rats. (15 min.) (Lantern.) Warren Andrew, Baylor University College of Medicine.

67. The regeneration of Eisenia foetida (Sav.) as determined by the lymphotrophic system. (15 min.) (Lantern.) Emil Liebmann, Department of Zoology, Duke University. (Introduced by L. H. Hyman.)
68. Imbedding without dehydration. (10 min.) Robert T. Hance, Duquesne University.
69. The conjugation of Vorticella microstoma. (15 min.) (Lantern.) Harold E. Finley, Morehouse College.
70. Double animals and multiple simultaneous mating in variety 4 of Paramecium aurelia in relation to mating types. (15 min.) (Lantern.) T. M. Sonneborn, Indiana University.
71. The effect of Trichomonas vaginalis on tissue culture cells. (15 min.) (Lantern.) M. J. Hogue, Medical School, University of Pennsylvania.

WEDNESDAY MORNING SESSION, DECEMBER 30TH

Business Meeting

The Annual Business Meeting of Section F, A.A.A.S., will be held Wednesday, December 30th, 12 M., Room 619, Hunter College. *The Annual Business Meeting of the American Society of Zoologists* will be held in the same room at 12:10 P.M., immediately following the meeting of Section F.

WEDNESDAY AFTERNOON SESSION, DECEMBER 30TH

Demonstration Program

Beginning at 2:00 P.M.; *General Demonstrations*, Room 720, Hunter College. (Beginning at 2:30 P.M.; motion pictures in Rooms 722 and 724, Hunter College.)

Motion picture demonstrations

Section A

105. Social organization and leadership among sheep. J. P. Scott, Wabash College. Motion picture to be shown at 2:30 P.M., Room 722, Hunter College.
106. Thiamin (vitamin B_1) deficiency in the cat. Guy M. Everett and Dietrich C. Smith, University of Maryland School of Medicine. Motion picture to be shown at 2:55 P.M., Room 722, Hunter College.
107. The biology of reproduction in rats. Edmond J. Farris, The Wistar Institute of Anatomy and Biology. Motion picture to be shown at 3:15 P.M.; Room 722, Hunter College.
108. Further observations on the sexual behavior of hormonally treated brown Leghorn fowl. L. V. Domm, David E. Davis and Ben Blivaiss, Whitman Laboratory of Experimental Zoology, The University of Chicago. Motion picture to be shown at 3:35 P.M.; Room 722, Hunter College.
109. The sexual behavior of Rana pipiens and its modification by brain injury in the male. Lester R. Aronson and G. Kingsley Noble, Department of Animal Behavior, The American Museum of Natural History. (Introduced by William Etkin.) Motion picture to be shown at 4:00 P.M.; Room 722, Hunter College.

(Motion picture demonstrations will be repeated upon request.)

Motion picture demonstrations

Section B

110. Return of vision four times in the same adult salamander eye repeatedly transplanted. L. S. Stone, Anatomical Laboratory, Yale University School of Medicine. (Introduced by W. O. Nelson.) Motion picture to be shown at 2:30 P.M.; Room 724, Hunter College.
111. Cine-photomicrographs of certain aspects of the motility of human spermatozoa. John MacLeod, Department of Anatomy, Cornell University Medical College. (Introduced by L. V. Domm.) Motion picture to be shown at 2:55 P.M.; Room 724, Hunter College.
112. Functional activity of the blood capillaries in the rat. C. G. Grand, Department of Biology, Washington Square College, New York. (Introduced by Robert Chambers.) Motion picture to be shown at 3:20 P.M.; Room 724, Hunter College.
113. Fixed persistence in the rat of spinal reflex patterns rendered extremely maladaptive by cross union of sensory nerves. R. W. Sperry, Harvard Biological Laboratories. (Introduced by A. B. Dawson.) Motion picture to be shown at 3:40 P.M.; Room 724, Hunter College.
114. Effects of colchicine experiments on chicken. Edna Higbee, University of Pittsburgh. Motion picture to be shown at 4:00 P.M.; Room 724, Hunter College.

(Motion picture demonstrations will be repeated upon request.)

General demonstrations

Section C

Cellular Physiology

115. Characteristics of human sympathetic ganglion cells cultivated in vitro. M. R. Murray and A. P. Stout, College of Physicians and Surgeons, New York.

Cytology

116. Water soluble micrological materials. Robert T. Hance, Duquesne University.

Embryology

117. An hereditary syndrome of abnormalities of spine, urogenital system and intestine in mice. S. Gluecksohn-Schoenheimer and L. C. Dunn, Columbia University.

General Physiology

118. The discharge of the penetrant (a nematocyst) by hydra. William A. Kepner and Herbert Taylor, Miller School of Biology, University of Virginia.
119. Asterias stomach tissue in anesthesia. Vera Koehring, Hunter College of the City of New York. (Introduced by Earl A. Dennis.)

Morphology

120. Stereobalanus canadensis (Spengel), a little-known enteropneustan from the coast of Maine. Edward G. Reinhard, Catholic University of America.

Protozoology

121. Demonstration of the cytosomic morphology of Paramecium polycaryum. E. M. Opton, Yale University. (Introduced by L. L. Woodruff.)

WEDNESDAY EVENING, DECEMBER 30TH, 6:30 P.M.

Zoologists' Dinner, East Ball Room of the Commodore Hotel.

Address by Dr. Warder Clyde Allee

Vice-President of Section F of the A.A.A.S., on

"Where Angels Fear to Tread"

(Open to all Zoologists and friends)

THURSDAY MORNING SESSION, DECEMBER 31ST

This session will be held in three sections, as shown below

Section A. *Endocrinology,* 9:30 A.M.

Room 619, Hunter College

72. Suppression of metamorphosis in the grasshopper Melanoplus differentialis. (15 min.) (Lantern.) Isabelle Weed Pfeiffer, Osborn Zoological Laboratory, Yale University.
73. Respiratory responses of normal and castrate goldfish to teleost and mammalian hormones. (15 min.) (Lantern.) Arthur D. Hasler and Roland K. Meyer, University of Wisconsin.
74. The effect of "high altitude" upon the pituitary and adrenal of the white rat. (15 min.) (Lantern.) Gerard R. Pomerat and Steven M. Horvath, Harvard University.
75. The anterior pituitary of the broody fowl. (10 min.) F. Payne, Indiana University.
76. Ovarian cysts in immature female cats following treatment with equine gonadotropin. (15 min.) (Lantern.) William F. Starkey and James H. Leathem, Allegheny General Hospital and Rutgers University.
77. Precocious development of secondary sex characters in juvenile Bufo fowleri by injections of various hormones. (15 min.) (Lantern.) Albert P. Blair, Laboratory of Experimental Biology, American Museum of Natural History.
78. Further observations on the effect of sex hormones on erythrocyte number in the brown Leghorn. (12 min.) (Lantern.) Elsie Taber and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.
79. Factors modifying the response of thyroidectomized rats to low temperature. (15 min.) (Lantern.) C. P. Leblond and J. Gross, Department of Anatomy, McGill University. (Introduced by R. Grant.)
80. Oxygen consumption of skin and hair growth after adrenalectomy in the white rat. (15 min.) (Lantern.) Earl O. Butcher, Hamilton College.

81. Effects of adrenal cortical hormones alone and in combination with prolactin on body and visceral weights in hypophysectomized pigeons. (15 min.) (Lantern.) Richard A. Miller and Oscar Riddle, Carnegie Institution, Department of Genetics.
82. The partial inactivation of desoxycorticosterone by the liver. (12 min.) (Lantern.) Wilburn J. Eversole and Robert Gaunt, Department of Biology, Washington Square College of Arts and Science, New York University.

THURSDAY MORNING SESSION, DECEMBER 31ST

Section B. *Cellular Physiology*, 9:30 A.M.

Room 620, Hunter College

83. The effect of oxalic acid, sodium oxalate and other calcium precipitating agents on wound healing. (15 min.) (Lantern.) Louis-Paul Dugal and Henri Laugier. University of Montreal.
84. Formulation of enzyme inhibitions: sulfanilamide, urethane, temperature, pressure. (15 min.) (Lantern.) Frank H. Johnson, Henry Eyring and R. W. Williams, Princeton University.
85. Characteristics of human sympathetic ganglion cells cultivated in vitro. (15 min.) (Lantern.) M. R. Murray and A. P. Stout, College of Physicians and Surgeons, New York.
86. Influence of the medium on radiation injury of sperm. (10 min.) (Lantern.) T. C. Evans and J. C. Slaughter, University of Iowa, G. Failla, Memorial Hospital, New York, and E. P. Little, Marine Biological Laboratory.
87. Changes produced in Arbacia sperm by immersion in irradiated sea water. (5 min.) (Lantern.) T. C. Evans, University of Iowa.
88. Inhibitory effect of caffeine on oxygen consumption in Arbacia eggs. (15 min.) (Lantern.) Ralph Holt Cheney, Long Island University.
89. The effect of respiratory inhibitors on the oxygen consumption of Paramecium calkinsi. (15 min.) (Lantern.) Edgar J. Boell, Osborn Zoological Laboratory, Yale University.
90. The action of various types of electric fields on the relative viscosity of the plasmagel of Amoeba proteus. (15 min.) (Lantern.) Clifford A. Angerer and Karl M. Wilbur, Ohio State University and Dalhousie University.
91. Fourteen-day rhythms in spiraling ratios of amebas and human leucocytes. (15 min.) (Lantern, motion picture projector.) A. A. Schaeffer, Temple University.

THURSDAY MORNING SESSION, DECEMBER 31ST

Section C. *Parasitology, Morphology and Ecology*, 9:30 A.M.

Room 718, Hunter College

92. A folliculinid carried by Limulus. (15 min.) (Lantern.) E. A. Andrews and T. C. Nelson, The Johns Hopkins University and Rutgers University.
93. The oyster crab, Pinnotheres ostreum, parasitic on the oyster. (15 min.) (Lantern.) Leslie A. Stauber, New Jersey Agricultural Experiment Station.

94. The urogenital system of the male frilled shark, Chlamydoselachus anguineus Garman. (15 min.) (Lantern.) Perry W. Gilbert, Cornell University. (Introduced by F. B. Hutt.)
95. The status of the modern amphibia among the tetrapoda. (10 min.) (Lantern.) F. Gaynor Evans, Duke University. (Introduced by A. M. Holmquist.) (To be presented by Wm. K. Gregory, Columbia University.)
96. Some unusual mammalian anomalies. (15 min.) (Lantern.) Bert Cunningham, Duke University.
97. Granular cells of the human juxta-glomerular apparatus. (15 min.) (Lantern.) F. W. Dunihue, Medical College, University of Vermont.
98. Concerning the group survival value of the social peck order. (15 min.) (Lantern.) W. C. Allee and A. M. Guhl, Whitman Laboratory of Experimental Zoology, The University of Chicago.
99. Tetraltera, a new homoeotic mutant in Drosophila melanogaster. (15 min.) (Lantern.) Claude A. Villee, University of North Carolina. (Introduced by R. E. Coker.)
100. The bearing of the genotype of extreme variants on selection. (15 min.) H. D. Goodale, Mount Hope Farm.

PAPERS PRESENTED BY TITLE

Cellular Physiology

122. Histological studies of vitamin E deficient testes of the fowl. F. B. Adamstone, University of Illinois.
123. The effects of vitamin E deficiency in the guppy, Lebistes reticulatus. H. W. Cumings, University of Illinois. (Introduced by F. B. Adamstone.)
124. Development of Arbacia eggs in caffeinized sea water. Ralph Holt Cheney, Long Island University.
125. Determination of polarity in Fucus eggs by gradients of dinitrophenol: a new method for establishing steep gradients across living cells. D. M. Whitaker and W. E. Berg, Stanford University.

Cytology

126. Amitosis in the malpighian tubules of Melanoplus differentialis (Acrididae). Leonard G. Worley, Brooklyn College and the University of Nebraska.
127. The mitotic index in the neural tube of the 48-hour chick as determined by the use of colchicine. T. M. Woodard and Sarah B. Estes, Vanderbilt University.
128. The time and rate of mitosis in corneal wound healing. L. B. Arey, Northwestern University Medical School.
129. On the nature and significance of grooved nuclei, especially in Brenner tumors. L. B. Arey, Northwestern University Medical School.
130. Endomitosis (?) in grasshopper serosa cells. T. N. Tahmisian, T. H. Allen and J. H. Bodine, State University of Iowa.
131. Cytological studies of hyperplastic livers in the rat. Norman M. Sulkin, State University of Iowa. (Introduced by H. W. Beams.)
132. Segregation and behavior of spermatozoa in Terebra dislocata. T. M. Woodard, U. S. Fisheries Bureau, Beaufort, and Vanderbilt University.

Ecology

133. Geographical distribution of the Folliculinidae. E. A. Andrews, The Johns Hopkins University.
134. Plankton organisms where fresh and salt waters meet. R. P. Cowles and C. E. Brambel, The Johns Hopkins University.
135. Studies on the black sediment in Back River, Baltimore, Md. C. E. Brambel and R. P. Cowles, The Johns Hopkins University.
136. Growth of the oyster drill, Urosalpinx cinerea, Say, feeding on four different food animals. James B. Engle, U. S. Fish and Wildlife Service, Milford Biological Laboratory. (Introduced by V. L. Loosanoff.)
137. Tolerance of the Gulf Coast snail, Thais floridana, to waters of low salinity. Victor Schechter, The City College of New York.
138. Comparative effects of low humidities on water loss and reproductive capacity of two species of saturniid moths. Daniel Ludwig, New York University, University Heights.
139. Life history of Hurter's spadefoot. Arthur N. Bragg, University of Oklahoma.
140. The habits of spadefoot toads in relation to their phylogeny. Arthur N. Bragg, University of Oklahoma.
141. The pyramid of life in some inland lakes of Wisconsin. Chancey Juday, University of Wisconsin.
142. Social behavior of the Western Banded Gecko. B. Greenberg, American Museum of Natural History and The State University of Iowa. (Introduced by P. L. Risley.)
143. Dominance, social order and territory in the lizard (Anolis carolinensis). B. Greenberg and G. K. Noble, American Museum of Natural History and The University of Chicago. (Introduced by W. C. Allee.)
144. Some aspects of the dominance-subordination relationship in Platypoecilus maculatus. James C. Braddock, The University of Chicago and Kansas State College. (Introduced by W. C. Allee.)

Embryology

145. Regeneration and transplantation in Clymenella torquata. Effects of pieces of posterior nerve cord inserted in the open, terminal wounds of worms cut to regenerate anteriorly. Leonard P. Sayles, The City College of New York.
146. Phenogenetic studies in the bar series of Drosophila melanogaster. Howard L. Gravett, Elon College. (Introduced by W. M. Luce.)
147. Effect of double matings on the fertility of a mutant of Drosophila melanogaster. R. C. Anderson and C. P. Oliver, University of Minnesota, Minneapolis.
148. Induced haploidy and triploidy in Californian Triturus. Donald P. Costello, University of North Carolina.
149. Neural crest transplantations between Hyla and Triturus embryos. Donald P. Costello, University of North Carolina.
150. Lacrimal duct determination and development in transplanted R. catesbeiana integument. O. M. Helff, New York University.

151. Colchicine and limb regeneration in larval Amblystoma. C. S. Thornton, Kenyon College.
152. The chromosomes of the alligator. Paul L. Risley, State University of Iowa.
153. The early development of the gonadal rudiment in the opossum (Didelphys virginiana). Olin E. Nelsen and Elisabeth Swain, University of Pennsylvania, and E. B. Morris Biological Farm of The Wistar Institute.
154. Variation in rabbit embryos. Allyn J. Waterman, Williams College.

Endocrinology

155. The control of metamorphosis in Hymenoptera. R. G. Schmieder, University of Pennsylvania.
156. Hormonal regulation of molting and some related phenomena in the crayfish, Cambarus immunis. Harold H. Scudamore, Northwestern University. (Introduced by F. A. Brown, Jr.)
157. Influence of the sinus glands upon certain metabolic changes associated with molting in the crayfish, Cambarus immunis. Harold H. Scudamore, Northwestern University. (Introduced by F. A. Brown, Jr.)
158. Hormonal influence on molting and the sexual cycle in the crayfish, Cambarus immunis. Harold H. Scudamore, Northwestern University. (Introduced by F. A. Brown, Jr.)
159. Vitamin B_1 and metamorphosis in Rana. E. D. Goldsmith, College of the City of New York, Washington Square College of Arts and Sciences, New York University, and R. F. Nigrelli, New York Aquarium.
160. Some effects of testosterone on the sexual pigmentation and other sex characters of the cricket frog (Acris gryllus). B. Greenberg, State University of Iowa. (Introduced by Paul L. Risley.)
161. Hormonal determination of sex behavior in normal and castrate second year juvenile herring gulls (Larus argentatus). Willis R. Boss and Emil Witschi, State University of Iowa.
162. The influence of some external factors on the ovarian cycle of the female starling. J. Wendell Burger, Trinity College (Conn.).
163. Ovarian hormones and spontaneous running activity in the female albino rat. William C. Young and William R. Fish, Yale University and Yerkes Laboratory of Primate Biology.
164. Variations in the sexual behavior pattern in the female guinea pig and rat. William C. Young, Yale University and Yerkes Laboratory of Primate Biology.
165. A case of complete hermaphroditism in the grass frog, Rana pipiens. J. Theron Illick, Syracuse University.
166. Testicular response in the English sparrow, Passer domesticus (Linnaeus), to gonadotropic hormone stimulation. Adolph R. Ringoen and John E. Allison, Department of Zoology, University of Minnesota.
167. Ambisexual nature of ovarian grafts in female rats (of prostate strain and non-prostate strain). Thomas Hernandez, State University of Iowa. (Introduced by Emil Witschi.)

168. Ovulation induced in Rana pipiens by bird pituitary preparations. Charles W. Creaser and Roman Harkaway, Wayne University.
169. Synchronized induction of ovulation and premature oviposition in the domestic fowl. R. M. Fraps, U. S. D. A., Beltsville, Md.
170. The occurrence of regression in ovarian follicles of the hen following administration of a luteinizing extract. R. M. Fraps and A. Dury, U. S. D. A., Beltsville, Md.
171. Certain effects of gonadotropic hormones on the developing ovary of the opossum. Olin E. Nelsen and Elisabeth Swain, Department of Zoology, University of Pennsylvania, and E. B. Morris Biological Farm of The Wistar Institute.
172. The effect of estrin on the function of corpora lutea in hypophysectomized rats. Warren O. Nelson and J. Walton Pichette, College of Medicine, Wayne University.
173. Age changes in the anterior pituitary gland of the mouse. Kathryn F. Stein, Elizabeth Caldwell and Jean Peters, Department of Zoology, Mount Holyoke College. (Introduced by Christianna Smith.)
174. The effects of thyroxin administration on the adrenals, thyroids and anterior pituitaries of non-pregnant and pregnant mice. A. Elizabeth Adams, Mary Medlicott and Marjorie Hopkins, Mount Holyoke College.
175. Factors influencing antihormone production. Albert S. Gordon, Department of Biology, Washington Square College of Arts and Science, New York University.
176. A preliminary study of changes in the blood of gray Norway rats following audiogenic seizures. William H. Cole, Eleanor H. Yeakel and Edmond J. Farris, Rutgers University and The Wistar Institute of Anatomy and Biology.
177. Effect of feeding two eggs from a colchicine injected hen to 1-day-old baby chicks. Edna Higbee, The University of Pittsburgh.

General Physiology

178. Variable factors of fertility in Lymnaea columella. Grace Springer Forbes, Barnard College, Columbia University. (Introduced by Henry E. Crampton.)
179. The effect of added amounts of vitamin B_1 on the life cycle of Lymnaea columella. Grace Springer Forbes, Barnard College, Columbia University. (Introduced by Henry E. Crampton.)
180. The digestion of collagen by enzymes. Irwin W. Sizer, Massachusetts Institute of Technology.
181. Islets of Langerhans in the pancreas of white rats. Walter N. Hess, Hamilton College.
182. The production and analysis of feather-chimaerae in fowl. Frank R. Lillie and Hsi Wang, Whitman Laboratory of Experimental Zoology, The University of Chicago.
183. The morphogenetic role of the dermal component of the papilla, and feather-forming capacity of the general follicular epidermis. Hsi Wang, Whitman Laboratory of Experimental Zoology, The University of Chicago. (Introduced by Paul Weiss.)

184. Observations on the light requirements of the weaver finch, Pyromelana franciscana. Marie Rollo and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.
185. The carotenoids of the display plumages of African bishop birds. Henry Kritzler, State University of Iowa. (Introduced by Emil Witschi.)
186. Relation of thyroid gland to plumage pattern and gonad function in the brown Leghorn male. Ben Blivaiss and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.
187. Relation of thyroid gland to plumage pattern and gonad function in the brown Leghorn female. Ben Blivaiss and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.
188. Feather succession in a case of hemi-lateral asymmetry. Mary Juhn, University of Maryland.
189. Feather pigmentation and leucosis in the brown Leghorn. Mary Juhn, University of Maryland.
190. The effect of thyroidectomy on spermatogenesis in the adult male guinea pig. Landrum B. Shettles and G. E. Seegar Jones, Departments of Obstetrics and Gynecology, The Johns Hopkins University.
191. The cycle of egg and sperm production in Amphiuma tridactyla Cuvier. Francis H. Wilson, Tulane University.
192. Seasonal changes in the reaction to the turtle's vagus. Dorothy Fetter, Helen C. Coombs, Brooklyn College, and F. H. Pike, Columbia University.
193. Comparison of the role of the afferent vagal proprioceptive fibers in respiratory control in birds and mammals. W. C. Randall and W. A. Hiestand, Western Reserve University and Purdue University.
194. Respiration of the Reptilia as influenced by the composition of the inspired air. D. E. Stullken, W. C. Randall and W. A. Hiestand, Purdue University.
195. The responses to light of various wave lengths by the periwinkle, Littorina litorea (L.). Dwight Elmer Minnich, University of Minnesota.
196. Correlation of photochemical events with the action potential of the retina. Verner John Wulff, State University of Iowa. (Introduced by T. L. Jahn.)
197. Intensity-EMF relations of the electroretinogram of beetles possessing a diurnal rhythm. Verner John Wulff and Theodore Louis Jahn, State University of Iowa.
198. The effect of temperature on the retinal action potential. Theodore Louis Jahn and Verner John Wulff, State University of Iowa.
199. The effects of a sudden decrease in temperature on the blood picture of cattle. Elaine T. Delaune and Roy L. Mayhew, Louisiana State University. (Introduced by Ellinor H. Behre.)
200. Observations on starfish, Asterias forbesi, exposed to sea water of reduced salinities. Victor L. Loosanoff, U. S. Fish and Wildlife Service, Milford Biological Laboratory.
201. Effects of different concentrations of plankton forms upon shell movements, rate of water pumping and feeding and fattening of oysters. Victor L. Loosanoff and James B. Engle, U. S. Fish and Wildlife Service, Milford Biological Laboratory.
202. A null reading photoelectric microdensitometer. Joseph G. Baier, Jr., University of Wisconsin.

Morphology

203. The Ostracod, Chlamydotheca unispinosa, reported from the United States. Catherine Dobbin Evenson, Smith College. (Introduced by Elizabeth S. Hobbs.)
204. Comparative study of the air sac system of birds. Allyn J. Waterman and H. P. Eddy, Williams College.
205. Further studies of the cortical fusi of human-head-hair shafts, and their application to legal work. Leon Augustus Hausman, New Jersey College for Women (Rutgers University).

Parasitology

206. Lymphocystis disease in Centrarchidae. J. Richard Weissenberg, Department of Histology, Middlesex University Medical School.
207. Leeches (Ozobranchus branchiatus) on fibro-epithelial tumors of marine turtles (Chelonia mydas). Ross F. Nigrelli, New York Zoological Society.
208. The parasites of the Salamandridae (Amphibia: Caudata). I. A. C. Walton, Knox College, Galesburg, Ill.
209. The parasites of the Salamandridae (Amphibia: Caudata). II. A. C. Walton, Knox College, Galesburg, Ill.
210. The parasites of the Salamandridae (Amphibia: Caudata). III. A. C. Walton, Knox College, Galesburg, Ill.

Protozoology

211. More mating types and varieties in Paramecium aurelia. T. M. Sonneborn, Indiana University.
212. Evidence for two distinct mechanisms in the mating reaction of Paramecium aurelia. T. M. Sonneborn, Indiana University.
213. Physiology of the ciliate Colpidium colpoda. II. The growth of C. colpoda in non-living media. William D. Burbanck, Drury College. (Introduced by W. C. Allee.)
214. Growth of populations in relation to size of the inoculum in cultures of Glaucoma piriformis. R. P. Hall and W. B. Cosgrove, New York University.
215. Mineral deficiency as a factor limiting growth of the ciliate, Glaucoma piriformis. R. P. Hall and W. B. Cosgrove, New York University.
216. The comparative in vitro effect of various sulfonamide drugs on Leishmania tropica. Alfred M. Elliott, Minnesota State Teachers College, Bemidji.

ABSTRACTS

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ABSTRACTS

PAPERS PRESENTED IN PERSON

Endocrinology (Sect. A)

1. Relative sensitivity to certain ovulation-inducing agents of first and subsequent follicles of clutch sequences in the hen. R. M. Fraps and A. Dury, U. S. D. A., Beltsville, Md. (Lantern; 12 min.)

In earlier studies on the induction of ovulation in laying hens (Fraps, et al., '42), injections were made to force rupture of follicles normally ovulating subsequent to the first of clutch sequences. It has since been found that first follicles of regularly recurrent clutch sequences are ovulated 8 to 15 hours prematurely following injection of a luteinizing extract from sheep anterior pituitary glands at approximately 1/20 the level required for equal ovulatory effect on other than first follicles of clutches at comparable prematurity.

The greater sensitivity of first follicles of clutch sequences is not a result peculiar to the effect of the luteinizing extract of sheep anterior pituitaries. Intravenous injection of progesterone at the level of approximately 0.1 mg. forces ovulation of first follicles of clutch sequences, while ovulation of subsequent follicles at comparable prematurities is not induced following injection of 100 times this quantity.

The time from injection of appropriate ovulation-inducing agents to rupture of first follicles is of the same order, circa 8 hours, required for forced ovulation of other than first follicles of clutch sequences.

The apparently differential lability of the two types of follicles at equal hours prematurity may reside either in inherent follicular differences or in differences in the endogenous hormonal environment. Results bearing on the relative contribution of these factors in explanation of the observed ovulatory differentials will be presented.

2. Studies on the reproduction of viviparous snakes. Douglas E. Bragdon, Harvard University. (Introduced by Leigh Hoadley.) (Lantern; 15 min.)

Conflicting evidence, reported by Clausen ('40) and by Rahn ('39), regarding the activity of the ovaries in maintaining young in the uteri of viviparous snakes led to this reinvestigation of the problem. Castration and hypophysectomy experiments were carried out, using species of both Natrix and Thamnophis. In both genera, young continued to develop when the animals were ovariectomized midway through pregnancy. Living and normally developed young were found in snakes hypophysectomized early in gestation and killed after as long as eight weeks. The young develop to full term and then apparently suffocate in utero. Castration or hypophysectomy interferes with the birth mechanism. Some are born dead and in other cases the products of decomposition kill the mother. Living embryos or their

membranes may exert an influence on the uterus, since generally uteri containing living embryos are thick and heavily vasculated in contrast to those containing resorbing embryos or none at all. Weights of eggs at various gestational stages and young at parturition showed a loss of weight during development, indicating that the embryos do not receive nourishment from the mother. Also, the total calcium content of the young at parturition is no more than that of the egg in early gestation. No appreciable amounts of estrogen were found in Natrix eggs by the Astwood six-hour assay method. The results indicate that the ovaries and the hypophysis are not essential for normal development of the embryos, but their removal interferes with the normal birth mechanism.

3. Effects of a neurophysiological stimulus on the breeding of albino rats.[1] Edmond J. Farris and Eleanor H. Yeakel, The Wistar Institute of Anatomy and Biology. (Lantern; 15 min.)

A study was conducted on male and female rats of three age groups to test the effect of a neurophysiological stimulus on breeding. Experimental rats were stimulated by sound from an air blast five minutes daily for five days a week continuously since weaning and throughout this experiment. The auditory stimulation frequently caused running attacks and convulsions.

In the younger groups (74–261 days old), 69.8% of the 63 experimental females became pregnant, while 39.7% of the 63 controls became pregnant. None of the older animals (300–414 days) produced litters.

88.6% of the young born to experimental females survived the first day, compared with 98.5% for controls. At weaning, 77.2% of experimental young and 88.6% of control young were alive. The differences in survival at birth and at weaning were statistically significant. No significant differences were found in the weights, sex ratio, or number of young at birth.

We conclude that exposure to air blasting resulted in more pregnancies, but probably depressed maternal behavior.

[1] Aided in part by a grant from the Samuel S. Fels Fund.

4. Studies of reproduction in the albino rat.[1] Edmond J. Farris, The Wistar Institute of Anatomy and Biology. (Lantern; 15 min.)

In studies of reproduction in the female albino rat, a method was developed to establish by running activity puberty, estrous cycle regularity, four stages of estrus, and menopause.

I. In Stage II of estrus (two hours after beginning of heat) 90% of the resistant female rats became pregnant when permitted to mate no more than twenty minutes. In Stage III (Willingness, ♂ and ♀) only 10% pregnancies occurred. In Stage I the female fought the male and in Stage IV the male was uninterested in the female.

II. By running activity after mating, pregnancy could be established within four to five days. In all cases in which the female became pregnant there was a reduction in total running when compared with the previous estrous cycle, and the regular cycles disappeared until the litter was weaned. In mated females that

[1] This investigation was aided in part by a grant from the Samuel S. Fels Fund.

did not become pregnant there was a marked increase in running during the following cycle.

III. It was established that ovulation occurred in Wistar albino rats 8 hours after the beginning of heat in mated females and slightly later in the non-mated. Spermatozoa were identified in the uterus promptly after ejaculation. However, the spermatozoa present in the uterus were never released to travel further until just before or at time of ovulation, when they were found in the oviduct and ovarian sac.

IV. The spermatozoa became immobile in uterus four hours after ovulation, and disappeared completely from the uterus within three days after ovulation.

5. The influence of age, season and pregnancy on the character of the reproductive cycle in 22 chimpanzees. William C. Young and Robert M. Yerkes, Yerkes Laboratory of Primate Biology. (Lantern; 15 min.)

Six hundred and fifty-three reproductive cycles showed differences associated with age, season and pregnancy which have not hitherto been recorded. The cycle is regarded as being composed of a preswelling phase, assumed to be a period of follicular quiescence; the phase of tumescence and genital swelling assumed to be the period of follicular growth terminated by ovulation; the phase of detumescence and genital quiescence, assumed to be the luteal phase; and menstruation.

During 8 years following the menarche the mean lengths of preswelling and follicular phases changed from ± 20 to ± 3 and from ± 26 to ± 18 days respectively. The luteal phase lengthened from ± 6 to ± 12 days. Menstruation changed from ± 4 to ± 3 days and the cycle from ± 50 to ± 35 days. The character of the cycle following pregnancy frequently resembled the first reproductive cycles after the menarche.

The only changes that can be attributed to season involved the preswelling phase and entire cycle. Both were longer during the period of shorter days and lower temperatures, October–March, than during April–September.

Variation was greater in the preswelling and follicular phases than in the luteal phase.

The length of the phases is a variable percentage of the cycle, but from the available data no certain relationship exists between such variations and reproductive efficiency measured by the ratios of fertile to sterile matings and of normal pregnancies to still-births and abortions.

The period of adolescent sterility was frequently less than a year, in 2 of 12 subjects it was less than 6 months.

6. Quantitative studies on hormonal sex reversal in larval anurans. Emil Witschi, Charles Foote, and Beatrice Mintz, State University of Iowa. (Lantern; 15 min.)

Continuation of experiments by various investigators (lit. Cold Spring Harbor Symposia X) reveals an extraordinary responsiveness of the sex glands of tadpoles to some hormones. Larvae of Rana sphenocephala, R. pipiens and Pseudacris triseriata raised from the time of hatching in water containing 0.01 mg. of testosterone propionate (Perandren, Ciba) per liter (concentration 1: 100,000,000)

develop and differentiate as males, irrespective of their genetical constitution. Secondary transformation of large larvae of R. clamitans, in which the presence of well developed ovaries had been ascertained by laparotomy, was obtained by weekly intraperitoneal injections of 0.025 mg. of testosterone propionate. The fact that a dose ten times larger gave an almost identical response indicates that the minimal effective dose may be considerably lower than any tried so far. In contrast to the usual findings in mammals the female sex hormones are much less effective than androgens. Estrone and estradiol dipropionate (Di-ovocylin, Ciba) cause ovarial development in genetical males at concentrations above 1 mg. per liter, but not below. Progesterone, dissolved in the water or injected into the body cavities (0.05 to 0.5 mg. per week) has no masculinizing quality.

7. Deposition of melanin in the sparrow's bill as a response specific to androgens. Carroll A. Pfeiffer, Charles W. Hooker and Arthur Kirschbaum, Yale University. (Lantern; 15 min.)

The effectiveness of local application as compared to systemic administration of androgen in inducing deposition of melanin in the bills of male and female sparrows was studied. Pigmentation was less readily elicited and less uniform in intact females than in castrated males, doubtless because of fewer pigment cells in the female. The minimum effective systemically administered dose of testosterone propionate, in either oil or alcohol, in castrated males was roughly 8 micrograms daily over a period of 10 days, while the MED of testosterone in alcohol applied locally to the bill was a total of 1 microgram, irrespective of whether distributed over 4, 8 or 16 days. Similarly, the minimum effective total dose of cis-androsterone was 1 microgram given locally in 4 days. The response to the locally applied hormone was a sharply defined jet black line at the skin bill junction. With minimal doses this line might be less than 1 mm. in length, and most frequently was located in one of three positions: at the lateral margin of the lower mandible, on the lateral pouch or along the nasal notch of the upper mandible. Local applications of progesterone and desoxycorticosterone acetate did not evoke pigmentation in the bill even when given in amounts 5000 to 2500 times by weight, respectively, the minimal dose of testosterone. Thus the sparrow's bill appears to respond only to androgens and to be unaffected by other hormones which are slightly androgenic as determined by other criteria.

8. The preparation and biological assay of relaxin. A. A. Abramowitz, F. L. Hisaw, L. H. Kleinholz, W. L. Money, R. V. N. Talmage, and M. X. Zarrow. Harvard University. (Lantern; 15 min.)

Relaxin was prepared from defatted sow corpora lutea by extraction with 2 per cent HCl. After neutralization and removal of inert material, the solution was saturated with ammonium sulphate and the precipitate containing the hormone filtered off. Before use, it was dissolved in water and dialyzed for two days.

Guinea pigs weighing 250–350 grams were ovariectomized and injected with 0.83 mgm. estradiol daily for 4 days. On the 5th day, groups of 12 animals each were injected with various dosages (in terms of gram-equivalents fresh corpora lutea) of relaxin. A sigmoid curve relating percentage response (definite relaxation of the symphysis pubis 6 hours following injection) to dosage was obtained.

A unit of relaxin activity was defined as that amount of hormone producing a response in 60% of the animals under the above conditions. One milligram of the preparation containing 15.5% nitrogen and representing 1 gram corpora lutea has consistently given a unit response.

The dose-response curve obtained with the relaxin present in the serum of pregnant rabbits was in general similar to that described above. The activity (about 12 units per cc. serum) can be concentrated by the addition of 1 volume ethanol to 1 volume serum and removal of the inert precipitate. The supernatant solution contains the hormone, which can be precipitated by 9 volumes of acetone.

No estrogen was found in 120 units of our relaxin preparation, and 500 units were negative when tested for progesterone.

9. Relation of progesterone to the formation of relaxin. F. L. Hisaw M. X. Zarrow, R. V. N. Talmage, W. L. Money, and A. A. Abramowitz, Harvard University. (Lantern; 15 min.)

Castrated female guinea pigs given 250 mg. estradiol on 4 consecutive days will show relaxation of the pelvic ligaments, in 42 to 67% of the cases, between 72 and 96 hours following a single injection of 5 to 10 mg. progesterone on the fifth day. Animals similarly treated after castration and hysterectomy do not relax. In neither case will 250 mg. estradiol alone cause relaxation. However, both castrated and castrated-hysterectomized animals, given relaxin, will relax within 6 hours even though conditioned by only 0.83 mg. estradiol daily.

Normal female rabbits in oestrus given 5 mg. progesterone daily for 9 days and rabbits on the ninth day of pseudopregnancy have about one unit of relaxin in each 5 cc. of blood serum. Castrated females conditioned by 1.66 mg. estradiol daily for 5 days and given 1 to 5 mg. progesterone daily for 5 to 9 days also have about one unit of relaxin in 5 cc. serum. During the last week of pregnancy 0.1 cc. serum contains about one unit. Relaxin appears in the blood of oestrogen-conditioned castrated females between 48 and 96 hours following a single injection of 10 to 20 mg. progesterone.

Relaxin was not found in blood sera of hysterectomized-castrated rabbits and castrated males that had been conditioned by 1.66 mg. estradiol daily for 5 days and given 5 mg. progesterone daily for 9 days, thus indicating that the uterus is indispensable for the formation of relaxin under the conditions of these experiments.

10. Effects of estrogen and testosterone on mammary glands of hypophysectomized rats. Samuel L. Leonard, Cornell University. (Lantern; 15 min.)

That estrogen and testosterone are incapable of stimulating mammary growth in hypophysectomized rats has been the generally accepted conclusion of recent work. However, it will be shown here that terminal end buds of the mammary glands of hypophysectomized rats can be stimulated by estrogen if (a) immature healthy rats not exceeding 70 grams body weight are used, (b) injections of 50 to 1150 gamma of estradiol are begun immediately after the operation, (c) the glands are examined 10 to 12 days after the operation. Under any other conditions, no end bud growth resulted.

Testosterone in dosages of 12.5 mg. for 10 days injected into 22 to 35 day-old hypophysectomized male rats either immediately after the operation or after 7–9 days, results uniformly in a thickening of the duct system of the mammary gland. This is caused by dilation of the lumina, hyperplasia and hypertrophy of the epithelial cells. Secretory material is seen in the ducts and can be traced coming from the duct epithelium. These findings will be discussed in the light of factors influencing mammary growth.

11. The structure, function and seasonal changes of the so-called "airsacs" of the sage grouse. Hermann Rahn, Floyd L. Clarke and James R. Simon, University of Rochester and the University and Game and Fish Commission of Wyoming. (Lantern and Motion Picture Projector; 15 min.)

During the spring season the male sage grouse exhibits an unusual elasticity and enlargement of the oesophagus and overlying skin. During the actual courting on the parade ground this oesophagus is intermittently inflated to a capacity of several liters. The structural changes which develop as well as the method of oesophageal inflation will be described. Preliminary experiments suggest that the seasonal changes of the oesophagus are dependent upon the male sex hormone. The active inflation and deflation of these so-called "airsacs" will be illustrated by motion pictures.

General Physiology (Sect. B)

12. On the genetic basis for hermaphroditism. John W. Gowen, Iowa State College. (Lantern; 15 min.)

A dominant gene located on the third chromosome of Drosophila melanogaster causes the insertion of parts of the male genitalia between the anus and ovipositor in flies which would otherwise be normal females. These changes extend to the reproductive tract and sex combs. The sex combs have less teeth of more slender dimensions but are distinct. The reproductive morphology includes gross changes in both ovaries, presence of testis, great alterations in the female sex glands and presence of those characteristic of the male.

Super females, with 3 sex chromosomes and 2 sets of autosomes and the tendency of these 3 X's to push the individual more toward the female type, are also carried toward the male by this gene. Thus far the changes noted are not so pronounced as those in the diploid female but are of the same type.

Intersexes, with one dose of the hermaphroditic gene, are also pushed toward the male type but not to as great a degree as the diploid.

The triploids display no internal evidence of the hermaphroditic gene but are sterile.

13. The effect of temperature on the expression of intersexuality in Daphnia longispina. Katherine Sanford, Allegheny College. (Introduced by A. M. Banta.) (Lantern; 15 min.)

The temperature of culture may influence the manifestation of intersexuality, both the expressivity and penetrance, in a genetically intersex clone of Daphnia

longispina. Parthenogenetic females reared from birth at low temperature produced more highly intersex offspring, exhibiting fuller expression of male secondary sex characters, than did their sibs cultured at higher temperatures. Observations of cultures at 15°C., 25°C., 29°C., and at 32°C. indicated that the degree of manifestation of intersexuality varies inversely with temperature and actually disappears at 32°C. Efforts to localize a temperature-effective period by series of transfers of the mothers revealed an influence of temperature during both the ovarian and embryonic stages of development, but no temperature effect after the prenatal sensitive period. The transfer experiments also furnished some evidence for a carry-over effect extending to offspring produced several instars after the transfer of the mother from a modifying temperature environment.

14. Relative growth gradients and transformed coordinates related in the analysis of growth. Oscar W. Richards and Arthur J. Kavanagh. Research Department, Spencer Lens Co., Buffalo. (Lantern; 12 min.)

Relative growth gradients have been expressed by the allometric equation, $y = bx^k$, wherein y is the size of the part and x that of the whole organism. Growth of the part is relatively greater, less or equal to that of the whole as k is greater, less than or equal to unity. The magnitude of the constant b depends on the moduli of the scales used for measuring x and y and has no biological significance. The exponent k is the ratio of the specific growth rates (dx/xdt)/(dy/ydt). Thompson has shown that the outline of an organism drawn on coordinate paper may be transformed into that of another organism by systematically deforming the coordinates. The changing shape of the growing organism depends on the rates at which the different parts grow. The two methods have been combined and the growth of the tobacco leaf will be used to illustrate the analysis. The rate and direction of maximum and minimum growth at selected points, the contours of specific growth rate in area, and regulation will be shown graphically. The method is general and may be used for three dimensional analysis of the growth of both plants and animals when sufficiently detailed measurements of growing organisms become available.

15. The normal changes in weight and water content during the life cycle of the wood-eating beetle, Passalus cornutus Fab. I. E. Gray, Duke University. (Lantern; 15 min.)

The changes in weight and water content from egg to adult have been shown for a wood-eating beetle. Weight, volume, and water content are approximately doubled during development of the egg. Larvae increase in weight about 1000%. On emergence of the adult there is a sharp drop in weight amounting to about one-sixth the maximum larval weight. The water content increases from 67% in the newly laid egg to 90% in the larva, to 93% in the late pupa. The percentage of water in newly emerged adults falls slowly and is correlated with increase in dry weight and with the slow sclerotization of the integument. In old adults the water content amounts to 67%.

16. Protyrosinase and a liquid-air interface. T. H. Allen, A. B. Otis, and J. H. Bodine, State University of Iowa. (Lantern; 15 min.)

Protyrosinase was obtained from grasshopper eggs by a fractional precipitation method. To vials of 9.0 ml. volume were added 2.5 ml. of 0.9% NaCl, 1.25 ml. of 0.2 M phosphate buffer of pH 6.8, and 0.5 ml. of protyrosinase. The vials were shaken on the arm of a hydrogen electrode shaker. During some 100 min. samples were removed and analyzed in a Warburg apparatus.

From 0.7 to 2.0 oscillations per sec. the change of protyrosinase into tyrosinase and decomposition products approaches an equilibrium and proceeds according to a first order reaction which between 24.6°C. and 2.6°C. has a Q_{10} of 1.19.

Saponin, although in itself without the power of converting protyrosinase, affects the composition of the equilibrium mixtures. In the absence of saponin the components are 0.1 tyrosinase, 0.3 protyrosinase, and 0.6 decomposition products; in 0.05%, they become 0.6 tyrosinase, 0.2 protyrosinase, and 0.2 decomposition products. Increasing saponin to 0.6% changes this to 0.1 tyrosinase, 0.7 protyrosinase, and 0.2 decomposition products. Octanol, dodecanol, and hexadecanol produce similar effects dependent upon the particular alcohol. These polar-nonpolar compounds do not ionize, hence do not activate protyrosinase as do alkyl sulfate ions, yet they seem to alter shaking effects.

The reaction order, the temperature characteristic, and the effect of unionized polar-nonpolar compounds seem to indicate that protyrosinase is converted into tyrosinase and decomposition products at a liquid-air interface.

17. Amylase activity of human parotid and submaxillary-sublingual saliva. E. Alfred Wolf and John D. O'Neal, University of Pittsburgh. (Lantern; 15 min.)

By new methods for separate collection of the two kinds of saliva and for determining amylase activity, eight individuals were tested from 10 to 24 times for each kind of saliva. The following results were obtained:

Amylase activity was higher in parotid saliva of all individuals tested; submaxillary-sublingual (s-s) amylase, expressed in per cents of parotid amylase had a value of 88% in one individual but for the rest was much lower, ranging from 37% to 3%. In seven cases, out of eight, low values of parotid amylase corresponded with high values of s-s amylase and, conversely, high values of the first kind with low values of the second, so that a negative correlation between parotid amylase and s-s amylase activity was apparent.

Individuals differed as to variability of salivary activity: one could distinguish between individuals of low, medium and high coefficients of variation. Rate and variability of salivary amylase activity may be as much a characteristic of an individual as other physiological and physical traits.

18. The effect of exogenous adrenalin on frog renal haemodynamics. Roy Philip Forster, Dartmouth College. (Lantern; 15 min.)

Adrenalin was administered subcutaneously to 17 large bullfrogs and its effect on renal blood flow, glomerular filtration rates and filtration fractions was studied utilizing clearance methods in 201 urine collection periods. Adrenalin (suprarenin

and adrenalin chloride) was injected in doses varying from 0.01 to 1.0 mg. into animals ranging in weight from 300 to 900 grams. Blood samples were taken directly from the ventricle and accurate urine collections were made from cannulated explanted ureters. Diodrast clearance was used to measure the renal blood flow and creatinine clearance to measure concurrent rates of glomerular filtration. In some of the animals tubular glucose reabsorption calculations were made to estimate the number of functioning glomeruli. Constant plasma concentrations of diodrast, creatinine and glucose were maintained throughout the experiments by steady infusion of these substances into the sub-buccal lymph sac using the dropping mercury technique.

Adrenalin in these experiments constricted both the afferent and efferent glomerular arterioles. The initial response observed following the injection of adrenalin is a depression of the renal blood flow, the glomerular filtration rate, the filtration fraction and the urine flow, thereby indicating afferent constriction. Following this general drop efferent constriction is indicated by a significant increase in the filtration fraction to a value well above the controls and by an increase in the rates of glomerular filtration and urine flow. The number of functioning glomeruli decreases during afferent constriction and steadily rises during the recovery period.

19. Responses of the capillary bed in the frog to adrenal cortical hormones. B. W. Zweifach and Robert Chambers, Department of Biology, Washington Square College, New York. (Lantern; 12 min.)

The effect of various adrenal hormones on the capillary circulation was studied by microscopic observations of the living vessels in the tongue and exposed mesentery of the frog. Whole adrenal cortical extract and two fractions, Compounds A and E, (supplied by E. C. Kendall), were tested by cardiac intraventricular injection. The possible site of action of the cortical compounds was determined by using the following functional reactions.

1. The first set of criteria were the effect of minimal trauma with microneedles which produce an increased permeability to the dye T-1824, the development of sticking of carbon and leucocyte to the endothelial wall and in some cases capillary stasis. None of the hormones, on injection, were found to protect against the development of these reactions. Likewise there was no enhancement of repair of locally traumatized vessel walls.

2. Another set of criteria were the hyperemic state of the capillary bed caused by exposure, rubbing with microneedles, or the topical applications of 1: 100 histamine, and the concomittant loss of vasomotion (a periodic narrowing and dilatation) of the arterioles and precapillaries. It was found that the injection of whole extract or of compound A produced the striking effect of restoring and augmenting the tone and vasomotion of these muscular vessels and converting the hyperemic beds to their original, restricted state of circulation.

No similar effects were obtained by the injection of compound E or of epinephrin.

20. The effect of Kendall's cortical extracts on kidney tubules in tissue culture. Robert Chambers and Gladys Cameron, Department of Biology, Washington Square College, New York. (Lantern; 15 min.)

Tissue cultures of active chick embryo kidney tubules were grown in media containing adrenal cortical extracts including the crystalline compounds A and E obtained by courtesy of Dr. E. C. Kendall of the Mayo Foundation. The presence of the whole cortical extract and particularly of compound E markedly enhanced the secretory activity of the proximal tubules. On the other hand, no effect was observed with the compound A.

Embryology (Sect. C)

21. Hormones in Drosophila. Dietrich Bodenstein, Columbia University. (Lantern; 15 min.)

It has recently been shown that a hormone produced by the ring gland controls the growth of the imaginal organ anlagen in Drosophila during the larval period, as well as the imaginal differentiation of these discs during pupal life. Whether growth or differentiation takes place is determined by the relationship between hormone concentration and tissue responsiveness. The question remained whether this hormone also controlled larval molting. This has been answered as follows: Drosophila larvae molt twice before they pupate. At each molt the larvae, together with their cuticula, shed their mouth parts. The mouth parts are characteristic in size and shape for each larval instar. In the experiments the mandibular hooks were used as criteria for molting. The heads of newly emerged larvae were transplanted (without brain) into the body cavity of mature larvae shortly before pupation. At the emergence of the host it was found that the transplanted larval heads had molted twice, for three pairs of mouth parts were present in each transplanted head part. When similar head parts were transplanted into the body cavity of adult male flies together with several ring glands, molting could also be induced, but failed to occur when the head was transplanted without ring glands into adult hosts. The ring gland is thus responsible for molting, growth and differentiation.

22. The early embryonic development of Amaroecium constellatum. Sister Florence Marie Scott, Seton Hill College. (Lantern; 15 min.)

Amaroecium constellatum produces heavily yolk-laden eggs. The first cleavage divides the egg unequally into a smaller right and a larger left blastomere. The second cleavage separates two larger anterior from two smaller posterior blastomeres. The third cleavage is latitudinal, producing four small micromeres and four large macromeres.

At the sixteen cell stage the embryonic areas may be distinguished. They correspond to the areas of the less heavily yolked forms, Styela and Amphioxus, consisting of an anterior chorda-neural and a posterior mesodermal crescent enclosing the large yolk laden endodermal macromeres. Gastrulation is accomplished by overgrowth of the ectodermal micromeres, involution of mesoderm first at the lateral lips and then at the ventral lip, and by an abortive attempt of the endodermal cells to invaginate.

The invagination of mesoderm and the process of overgrowth proceed from the lateral and posterior borders of the yolk-impacted blastopore. The smaller cells on the right side divide more rapidly than the cells on the left side. This acceleration in division produces an asymmetry in the lip of the blastopore which closes from right to left. The neural plate grows posteriorly toward the left following the mesoderm as the blastopore closes, thus initiating the asymmetry of the neural tube in Amaroecium. The chordal cells are inflected under the neural cells and the definitive endoderm enclosed by the approximation of the ventral and dorsal lips of the blastopore. There is no archenteron.

23. STUDIES ON THE DEVELOPMENT OF THE KIDNEY OF THE EEL. Raymond Haringa and Charles G. Dobrovolny, Biological Institute, University of New Hampshire. (Lantern; 10 min.)

In the elver the pronephros consists of a pair of nephrons, the Malpighian bodies of which are located above the heart and have no communication with the body cavity. The mesonephros, located far posteriorly, is largely caudal to the anus. The blood of the glomeruli of both kidneys is arterial, that of the tubules venous. The pronephric tubules are straight except in the region of the distal convoluted segment.

The same histologically differentiated regions are found in both the nephrons of the adult eel and in the pronephric tubule of the elver. The brush border is higher in the second portion of the proximal convoluted segment in the elver while in the adult it is higher in the first portion. The transition from the distal convoluted segment to the collecting segment is abrupt in the adult nephron. In the elver the transition is more gradual and the distal convoluted segment is incompletely differentiated.

The nephrons of young elvers are simpler in structure than those of slightly older ones in having a low or rudimentary brush border in the proximal convoluted segment, a shorter, more undifferentiated distal convoluted segment, and less vascular glomeruli. These differences are associated with the transition from a marine to a fresh-water environment which the elver makes at this time.

The pronephric tubules do not persist long after the eel attains a length of 9 centimeters. Degeneration of the tubules may begin in 6 centimeter elvers. The epithelium disintegrates and is resorbed while the connective tissue sheath persists in various regions to form blood channels.

24. THE FORMATION OF THE BLASTODISC IN THE EGG OF THE ZEBRA FISH, BRACHYDANIO RERIO, ILLUSTRATED WITH MOTION PICTURES. Warren H. Lewis, The Wistar Institute, and Edward C. Roosen-Runge, Brown University. (Lantern, motion picture projector; 15 min.)

The fertilized egg is nearly spherical. The animal pole soon flattens. If one assumes that the outer hyaline layer of the egg is in the gel state it would, like the superficial gel layer of the amoeba or any other cell automatically exert contractile tension. An increase then in the viscosity or thickness of this layer at the animal pole which contains the pronuclei would account for this flattening. The blastodisc soon begins to appear over the flattened area. The continuous contractile tension of the superficial gel layer surrounding the yolk forces relatively clear

cytoplasm through the flattened area to form the blastodisc. The flattened area acts as a sieve and holds back the yolk granules. After an apparent period of rest, the continued contraction of the superficial gel layer produces a rather sudden streaming of fluid endoplasm and cytoplasm is forced out through the sieve to produce the rapidly enlarging blastodisc. The streaming subsides immediately after the first cleavage but reappears and subsides on a smaller and smaller scale with the next three divisions. The separation of the blastodisc from the yolk is now complete.

The formation of the blastodisc is thus analogous to the formation of the hyaline caps of the amoeba and of the slime mold and is due to the same fundamental factors, namely, the contractile tension of a superficial gel layer which forces the more fluid endoplasm through a localized porous layer.

Aided by a grant from the American Philosophical Society.

25. Effects of different degrees of polyploidy on viability, growth, and metamorphosis of larvae of the newt, Triturus viridescens. Gerhard Fankhauser and Rita C. Watson, Princeton University. (Lantern; 15 min.)

During the past five years a considerable number of polyploid larvae of Triturus viridescens were raised in the laboratory. Some of these were spontaneous polyploids developed from untreated eggs. The majority of the triploids were produced experimentally by treatment of fertilized eggs with cold or heat.

The development of three spontaneous haploid larvae came to a standstill shortly after hatching. The viability and growth rate of triploid larvae were normal. The time from fertilization to metamorphosis (first molt) varied from 77 to 115 days for 56 diploid animals of different groups. Thirty triploid larvae reached the first molt from 3 to 15 days later than the diploids. The single tetraploid larva found so far grew more slowly than the controls. It entered motamorphosis 58 days later than the diploids of this particular group, at a time when it had reached nearly normal size.

Of five pentaploid larvae three began to feed but soon showed a rapid decline in reactivity. One developed normally but more slowly than the controls to an early hind limb-bud stage in which it was preserved. One was raised beyond this stage but remained more and more behind the controls until growth ceased altogether at a time when the larva showed the first signs of approaching metamorphosis. It remained in this dwarfed condition for several weeks and died at the age of 170 days.

In Triturus, viability and growth rate thus are lowest for haploids, normal for triploids, but decrease again with higher chromosome numbers.

26. The origin of subcutaneous melanophores. Hans Elias, Elizabeth Quigg and Edward Sauris. Middlesex University. (Lantern; 5 min.)

It has been determined by Holmes ('14), DuShane ('35), Raven ('36), Bytinski-Salz ('38), Hilde Fischer ('38), and Ris ('41) that epidermal and cutaneous (i.e., adepidermal, subepidermal, and intracutaneous) melanophores derive from the neural crest. It has been the object of the present paper to determine whether this is true also for the subcutaneous melanophores, present in Anura. Extirpations, transplantations, and explants of neural crest of Rana

pipiens have been made; and it has been determined that the Anuran neural crest can give rise to subcutaneous melanophores. But if the neural crest is removed, other parts of the embryo can substitute it in the production of subcutaneous melanophores. Experiments to determine which parts can substitute the neural crest in its melanophore-producing capacity are still going on.

27. Sex reversal and the genetics of sex determination in the axolotl (Amblystoma mexicanum). R. R. Humphrey, Department of Anatomy, University of Buffalo. (Lantern; 15 min.)

The offspring of an axolotl female experimentally converted into a male and mated with normal females, included 26% males. If the female axolotl were homozygous for sex (XX), only females could have been produced in such matings. If, instead, the female were heterozygous (ZW), the F_1 offspring of a reversed female (1 ZZ + 2 ZW + 1 WW) would include 25% males (ZZ), assuming that the WW zygotes developed as viable females. Such females (genotype WW) when mated to normal males (ZZ) should produce no male offspring but only heterozygous females (ZW).

Sixteen F_1 females (daughters of the reversed female) have now produced offspring. Ten of these have produced males in the usual numbers (approximately 50% of over 650 F_2 reared). The remaining six have produced only female offspring (626 F_2 reared), whether mated with distantly related males, with brothers, or with their father, the reversed animal. Four F_1 males have produced male offspring in the usual numbers in at least one mating each, and are therefore judged to be identical in genotype with other axolotl males.

Since the F_1 offspring of the reversed female included 26% males, and since approximately one-third of the F_1 females have produced only female offspring, the normal axolotl female must be heterozygous (ZW) in genotype and the WW combination must result in a viable female. Such females are indistinguishable from those of the usual genotype and must be identified by ascertaining the all-female character of their offspring.

28. Growth of the frog kidney carcinoma when transplanted to the tails of normal and permanent tadpoles. Robert Briggs and Ronald Grant, Lankenau Hospital Research Institute and McGill University. (Lantern; 15 min.)

Kidney carcinoma tissue from adult Rana pipiens was implanted into the dorsal mesenchyme of the tails of young tadpoles (appr. 20 mm. total length). In normal tadpoles the tail implants grew well and maintained the typical adenocarcinoma structure until the hosts grew to be about 50 mm. in total length. At this time the tumors began to regress. In most cases regression of the tumor was complete by the time metamorphic absorption of the tail commenced (at appr. 65 mm.). Regression apparently was not due to failure of the blood supply to the tumor, or to lack of space for growth in the dorsal mesenchyme. We considered that pre-metamorphic changes in the tail might be responsible for the regression of the implanted tumor, and tested this idea by implanting bits of the tumor into the tails of non-metamorphosing tadpoles (hypophysectomized, and throidectomized). In these animals the tumor grew and regressed just as in the normal

control hosts, showing that tumor regression is not due to changes in the host connected with metamorphosis. It may be that regression results from a tissue specificity which develops independently of metamorphosis in large tadpoles.

29. Stimulation of the hen's egg by ultraviolet. Alexis L. Romanoff, Cornell University. (Lantern; 10 min.)

After a successful stimulation of fresh hens' eggs by x-ray with the increase in the potential differences between the blastoderm and the albumen (Romanoff and Bless, Proc. Nat. Acad. Sci., 28: 306–311, 1942) attempts have been made to repeat the stimulation by ultraviolet rays. Of the two wave-length regions studied, 3100 Å and 3650 Å, the latter region gave nearly twice as much stimulation with the increase of bioelectric potentials, about 60% above control, as the former one. The maxima of stimulation were attained within narrow ranges of radiant energy (erg./mm.^{2}). Small doses, preceding the stimulating region, had no effect, while large doses of ultraviolet beyond the region inhibited the development and diminished the potential difference.

30. The development and regression of the primitive streak in the explanted blastoderm marked with carbon particles. Nelson T. Spratt, Jr., The Johns Hopkins University. (Lantern; 15 min.)

Movements of cells in the epiblast associated with the formation and elongation of the streak and with its subsequent regression have been traced by placing on them small quantities of powdered blood charcoal.

Just prior to and during the formation of the short streak there is extensive postero-medial movement (toward the site of streak origin) of lateral areas of the posterior two-thirds of the pellucid area, but only a slight anterior movement of cells in the midline. A mark placed on the anterior end of the short streak remains in position as the streak elongates in front of it (38 cases). This indicates that the initial elongation is the result of a progressive forward proliferation and invagination of mesoderm in front of the original anterior end of the streak. After the streak has reached the center of the pellucid area subsequent elongation is by growth between its two ends resulting in an extensive backward movement of the original posterior end as the pellucid area changes shape (60 cases). During the development of the streak there is pronounced medial and postero-medial movement of lateral marked areas into the streak, some carbon particles invaginating with the mesoderm. The following changes in position of marks accompany regression of the definitive streak: Marks placed on the node and on the middle of the streak remain equidistant but come to lie more posteriorly with respect to immobile reference marks. Some particles invaginate with the chorda. Marks lateral to the streak move postero-medially into the streak.

31. Effects of localized x-radiation on regeneration of the Urodele limb. E. G. Butler and J. P. O'Brien, Princeton University and De Paul University. (Lantern; 10 min.)

Experiments were designed to test whether, by use of appropriate technique, regenerative capacity can be suppressed in a very restricted region of an organ

and at the same time retained in closely adjacent regions. Larvae of Eurycea bislineata, 25 to 50 mm. in length, were used as experimental material. A portion of a hind limb, 2 to 2.5 mm. in extent, including the knee-joint and the regions immediately distal and proximal to it, was exposed to x-rays; the remainder of the animal was completely shielded from the radiation by lead. When such a limb was amputated through its distal unradiated region, regeneration ensued normally. A second amputation, through the radiated region, resulted in complete absence of regenerative activity. When, after a period of nine weeks, the limb was again amputated, this time through its proximal unradiated region, normal regeneration followed. In these experiments, therefore, a limb, having been incapable of manifesting any regenerative capacity, after amputation through the radiated region, was enabled to express this capacity only when a new level of amputation was established through the proximal unradiated part of the limb.

These results provide clear-cut evidence that the regeneration process in certain essential features is local in nature. The "building blocks" used in the establishment of the blastema, and eventually of the new regenerate, have an exclusive and restricted origin at the site of amputation.

32. The effect on the embryos of x-radiation of the male rat. Margaret Henson, New York University. (Introduced by Roberts Rugh.) (Lantern; 15 min.)

Male rats of known pedigree were exposed to doses of x-radiation from 100 r to 3000 r prior to mating with normal, unirradiated females which had previously been tested with normal, unirradiated males. Exposure of males to 100 r resulted in only slight reduction in litter size but a 50% reduction in survival value of offspring. This effect wore off within a month. Exposure to 1000 r resulted in litters of one-third normal size and poor survival were obtained, these results being further exaggerated in matings made a month later. Implantation and resorption sites were found in these latter animals. Exposure of males to 3000 r caused loss of activating and fertilizing power of spermatozoa within 48 hours. Sterility of male rats exposed to 500 r and 1000 r occurred in 3 weeks from the time of exposure.

33. Rib ossification patterns in 12- and 13-ribbed rabbits. Inez Baumgartner, Brown University. (Introduced by Paul B. Sawin.) (Lantern; 15 min.)

The rib ossification in 62 embryos from a family of rabbits producing 98.7% 12-ribbed progeny was compared with that in 65 embryos from a family producing progeny of which 80% or more were 13-ribbed. The embryos were removed at each of four stages after coitus (17 days, 11 hours; 17 days, 15 hours; 17 days, 18 hours; 18 days, 11 hours), macerated in KOH, stained with alizarine and cleared in glycerine; the ossified portions of the ribs were measured with an ocular micrometer attached to a binocular dissecting microscope.

The general process of ossification agreed with that found in rats, mice, swine and man in that it began on the central ribs, progressed in a dorso-ventral direction in each rib and in an antero-posterior direction to complete the rib complement. Fundamental differences were observed in the ossification patterns of the two families. In the 12-ribbed group ossification began earlier, originated more

anteriorly, and progressed more rapidly on the anterior ribs; whereas, in the 13-ribbed family ossification proceeded more slowly in the early and late stages but was greatly accelerated during the period between the 2nd and 3rd stages, and generally progressed more rapidly on the posterior ribs. The fact that the 13-ribbed and the 12-ribbed embryos occurring in the 13-ribbed family show the same ossification pattern suggests that the ossification patterns are familial and that variation in rib number within the family is due to something else — extra-genetic causes or factors affecting preliminary processes.

General Physiology (Sect. B)

48. Acidosis in the rabbit during "gravity shock." William H. Cole, James B. Allison, James H. Leathem, William L. Nastuk, and John A. Anderson, Rutgers University. (Lantern; 15 min.)

Rabbits suspended by the ears, without anesthesia, became unconscious in an average of one hour. Struggling and convulsions were rare. The shallow rapid breathing of normal animals became deeper and slower upon hanging and a nearly constant rate of seventy-five per minute continued to unconsciousness. Rectal temperatures increased from an average of 39.5 to 40.1°C. When taken down the animals were limp, but immediately began to recover as the breathing rate returned to normal. Within twenty minutes, posture and behavior appeared normal, although the animals remained "tired" for an hour longer. About 30% died later, up to 12 hours, while the others recovered completely. Autopsies revealed congestion of blood vessels in the viscera, frequent enlargement of the whole or part of the spleen, and of the adrenal glands. No edema was seen. The pH of heart blood before hanging varied from 7.39 to 7.51, averaging 7.44, but at the time of unconsciousness varied from 6.81 to 7.34, averaging 7.08. Those with a blood pH below 7.0 died. The carbon dioxide and oxygen content of whole blood averaging 16 and 6.5 millimoles per liter in normals, dropped to 10.2 and 4.2 respectively. Lactic acid, pyruvic acid and inorganic phosphate increased markedly in the plasma of "shocked" animals, the total increments being sufficient to account for the acidosis.

Hanging of the rabbit caused posterior blood stasis, consequent tissue anoxia, and an acidosis due to lactic acid, pyruvic acid and phosphates resulting from metabolic disturbances.

49. Alteration of frequency of pacemaker nerve cells by imposed direct current. T. H. Bullock, Yale University. (Lantern; 15 min.)

Considerable indirect evidence has accumulated in support of the theory (Gerard, Gesell) that the frequency of firing of the nerve cell depends upon the DC field (electrotonic, somatic, or steady state potential) imposed on it. Direct evidence of a change in frequency during passage of a small constant current seems, however, to be lacking, although various effects of polarizing complex nerve centers have been described (brain wave amplitude changes; anesthesia; excitability changes).

Polarization of the cardiac ganglion of Limulus results in an abrupt, maintained, reversible increase in frequency of heart beat and changes in wave form and duration of the electrocardiogram. The effect is exerted on the ganglion, not

on peripheral nerve or muscle. A decrease in frequency was not obtained. This may be interpreted in at least two ways. If there are potential pacemaker cells oriented in all directions, and if orientation of the DC with respect to the nerve cell determines frequency of discharge, any given imposed current would find some cell properly oriented for acceleration. If the magnitude of the DC determines frequency regardless of macroscopic or axial orientation then again it would not be expected that a decreased rate could be obtained by imposing current. Wave form and other changes can be interpreted in terms of altered pattern of firing of lower order neurons, shift of pacemaker and effects of the DC on frequency of firing within each burst.

50. Effect of direct and diffuse light on the copepod Acartia tonsa. William Schallek, Harvard University. (Introduced by George H. Parker.) (Lantern; 15 min.)

Vast numbers of plankton organisms move toward the surface of the water at night and sink downward during the day. Laboratory tests show that many of these animals are positively phototropic. The following study was made to explain why organisms which move away from daylight in the sea move toward the light in the laboratory.

When the copepod Acartia tonsa is placed in tall glass cylinders exposed to window light, the normal behavior continues. Under artificial light the animals continue to move up in the dark and down in the light, but only as long as the illumination is oblique to the axis of the cylinder. When the lamp is overhead the animals reverse their behavior and swim up toward the light. Photometric measurement shows that this behavior depends upon the angular distribution of the light. The overhead lamp provides a direct illumination, coming from one direction only, while the oblique light is more diffuse, coming almost equally from several directions. In direct illumination A. tonsa reacts phototropically, swimming toward the light. In diffuse illumination it does not orient to the light but sinks passively downward.

Measurement of light distribution in nature shows that it is even more diffuse than in the cylinder. The downward movement of A. tonsa in diffuse light in the laboratory accords with its downward movement in the ocean during the day. Its reaction in the highly concentrated light in which phototropism experiments are usually conducted has no bearing on its behavior in nature.

51. Spacial relations of ommatidia in insects and differential sensitivity to moving visual stimuli. Ernst Wolf, Harvard University. (Lantern; 12 min.)

Dragonfly larvae (Anax) and flies (Calliphora, Musca), if held in a fixed position while stimulated visually by a surrounding vertically striped rotated cylinder, respond with characteristic leg movements or postures. Ommatidial areas of different sizes and locations on one or both eyes are tested for their responsiveness to anterior-posterior and posterior-anterior motion of the striped pattern in relation to the animal's axis. (a) There is a decrease in sensitivity from front to back for both directions of stripe motion; (b) the sensitivity also decreases with decreasing visual area; (c) unequal areas on the two eyes can enhance or inhibit the response. (d) When determining for Anax threshold intensities of light for

response to flicker produced by stripes passing from front to back, or from back to front on one eye, the threshold intensity for anterior-posterior motion is always lower than for response to motion in the opposite direction. With limited visual areas, however, the most posterior portion of the eye seems again to respond more readily to stimuli passing from back to front. The ommatidia therefore and their capacity to summate stimuli to the point of giving rise to a threshold response are organized in such a way that a visual response threshold is reached at lower intensities when a stimulus passes anterior-posteriorly than when in the opposite direction, except for the most backward located ommatidia which will also readily respond to a posterior-anterior motion but with a higher threshold.

52. Reestablishment of visuomotor coordinations by optic nerve regeneration. R. W. Sperry, Harvard Biological Laboratories. (Introduced by A. B. Dawson.) (Lantern; 15 min.)

In 58 adult newts (Triturus viridescens) the eyeball was rotated 180 degrees and the optic nerve was severed in different ways designed to favor random reassortment of the regenerating fibers. Recovery of reactions to visual stimuli occurred within 28 to 95 days. Reversal of vision conforming to the rotated position of the retina was clearly manifest in all cases, as indicated by deliberate reversed reactions, erroneous spatial localization, and other abnormalities of visuomotor coordination directly correlated with rotation of the retinal field. Thus the original functional values of various retinal loci were systematically restored when the fibers had reestablished connections with the visual centers. The reversed reactions persisted without modification by experience despite their decidedly maladaptive character.

These results indicate that the original functional properties of various retinal points are reestablished without aid of functional adaptation by the growth process itself, and furthermore that the optic fibers from different retinal loci are distinguished from each other in the centers according to location of their ganglion cell bodies in the retinal field, probably through differential physico-chemical properties induced in them by differentiation of the optic cup in development. The effect of this optic fiber specificity in determining orderly functional relations in the centers can be accounted for either on the basis of the Resonance Principle or alternatively in accordance with the author's hypothesis of peripheral induction of central connection specificity.

53. The effects of muscle length and shock intensity on muscular latency-relaxation. Alexander Sandow. Washington Square College of Arts and Science. (Lantern; 5 min.)

The latency-relaxation, LR, was recorded with a piezo-electric, cathode-ray oscillographic technique. All experiments were performed on the frog sartorius at about 20°C. Measurements were made of the time from the instant of stimulation to the beginning of the relaxation, L_R, and to various later instants signifying latency stages in the development of positive tension, collectively designated by L, and of the magnitude of the relaxation, R.

As shock intensity applied to a curarized muscle increases: L_R and L decrease, with minima of about 1.50 and 2.50 ms., respectively, and R increases to a maximum

of about 25 μ. Changes in R, especially, continue even for supermaximal shocks. In normal muscles essentially the same changes occur, but modified by the interactions of directly and indirectly stimulated muscle fibers; R is at minimum for shocks just threshold for muscle fibers.

As initial length increases (maximal shocks throughout) L and R each show a maximum and L_R a minimum at the length for maximal developed tension.

Conclusions: (1) Since the p.d. of the muscle action potential varies independently of R, and since no LR is obtained in an excited nerve trunk, LR is not an aspect of the ordinary excitation and conduction mechanisms. (2) Since R increases while muscle tension and action potentials are maximum, LR does not follow an all-or-none behavior. (3) In view of (1) and (2), and since at shorter lengths, L decreases with decrease in length, it is inferred that LR is a passive change due to decrease in the elastic modulus of the contractile substance.

54. The effect of tetanus on muscular latency-relaxation in normal and iodoacetate-poisoned muscles. Alexander Sandow. Washington Square College of Arts and Science. (Lantern; 5 min.)

The LR changes of sartorius muscles subjected to maximal twitch shocks, were determined before a maximal tetanus and at intervals during the subsequent recovery period.

In the normal muscle tetanus causes an immediate drop in the values of L and R, and these decreases are reversed during recovery over a period of 15–30 minutes. L_R remains constant. The magnitude of the changes is proportional to the length and frequency of the tetanus. The cycle of changes due to tetanus, excepting complete recovery, can be repeated several successive times at 5 min. intervals.

In the IAA muscle, tetanus causes the immediate decreases in L and R, and no change in L_R, as in normal muscle, but the recovery reversals do not occur. When the IAA muscle is tetanized at 5 min. intervals, each new tetanus causes further depression of R and L, until pronounced rigor and greatly diminished tension appear, when L, and L_R also, markedly increase.

In view of chemical and pH changes known to be associated with tetanic activity the following inferences are made. (1) L and R are decreased in proportion to a certain depletion of creatine-phosphate and increase in pH. (2) When, due to complete depletion of CP, adenosine-triphosphate is not resynthesized, L and L_R rise, while R decreases further. (3) Along with moderate depletion of CP and increase of pH, the rate of ATP decomposition would increase, and this is supposed to account for the increased rate of tension development as is evidenced in the shorter L values.

55. On the significance of muscular latency-relaxation. Alexander Sandow. Washington Square College of Arts and Science. (Lantern; 5 min.)

The conceptions developed in the above reports are confirmed and extended by the results of Englehardt that myosin is adenosine-triphosphatase, and that myosin threads, in catalyzing the hydrolysis of ATP, suffer a reduction in elastic modulus, a change not caused by any other muscle metabolite. In view of these

facts, it is assumed that the latency-relaxation is attributable to the interaction in live muscle of myosin and ATP. Since the actual relaxation has a fleeting existence just prior to contraction of only about 1.5 ms., it is inferred that it is a mechanical indication of the formation of an intermediate complex of enzyme and substrate, during whose existence energy for contraction is tranferred from ATP to myosin. The duration of the latent period would thus be determined by the rate of hydrolysis of ATP, a conclusion independently drawn from the effects of activity on the latencies for tension development. And since the resynthesis of ATP is coupled to the hydrolysis of creatine-phosphate, the rate of this reaction would determine the length of the refractory period.

Evidence supporting these suggested mechanisms may be found in the effects of pH on the rates of ATP and CP hydrolysis, respectively, and on the following features of muscular activity: mechanical output, latent period, refractory period, Hill's dynamical constant, b, and the rate of myosin ATP-ase activity in vitro.

The above discussion does not exclude the possibility that post-contractile relaxation is also dependent on an interaction of myosin and ATP. Some evidence exists that this may occur.

56. The effects of insulin on the responses of the frog's heart and rectus abdominis to acetylcholine. John H. Welsh, Harvard University. (Lantern; 15 min.)

The responses of the isolated frog's heart and rectus abdominis muscles to acetylcholine have been studied following experimental modification of glucose metabolism in the intact animal. Frogs maintained at two constant temperatures (15° and 25°C.) were injected with one unit of insulin. After intervals of 6, 12, 24 and 48 hours individuals were killed and concentration-response curves of the isolated heart and rectus abdominis to acetylcholine obtained at 20°C. At the same time blood sugar and muscle (gastrocnemius) glycogen determinations were made. Controls were run with both experimental groups.

The contractions of the recti abdominis of frogs maintained at 15°C., 6, 12, and 24 hours after insulin, were greater than those of control frogs, while 48 hours after insulin they were less. The hearts of these animals showed less depression to acetylcholine 6, 12 and 24 hours after insulin but greater depression 48 hours after insulin.

The contractions of the recti abdominis of frogs maintained at 25°C., 6, 12 and 24 hours after insulin, were all less than the controls while the depression of the hearts by acetylcholine was greater. Observations 48 hours after insulin were not possible at this temperature due to the death of the frogs from extreme hypoglycaemia.

While the results indicate that the responses of the frog's heart and rectus abdominis to acetylcholine may be modified by the administration of insulin and by temperature, results from the determinations of blood sugar and muscle glycogen suggest that the rate of utilization of glucose is more important than the levels of blood sugar or muscle glycogen at any given time.

57. The structure, development, and discharge of the penetrant of Pelmatohydra oligactis (Pall). William A. Kepner, Bruce D. Reynolds, Lewis Goldstein, and Herbert Taylor, Miller School of Biology, University of Virginia. (Lantern; 15 min.)

The penetrant is entirely a secretion-product of the cnidoblast. Its parts are: an oval operculate capsule; an axially disposed spinneret; and a rod of colloid-like substance that forms six spiral loops that wind loosely about the spinneret. The spinneret and its shaft constitute an inverted tapering tube plugged, at its fundus, by an end of the spiral rod. The mouth of the inverted shaft of the spinneret is closed, in an undischarged penetrant, by the capsule's operculum.

With the release of the operculum, water rushes into the lumen of the spinneret making contact with the end of the spiral colloid-like rod at the fundus of the spinneret. The spiral rod is now reduced to a magma and the internal pressure is raised. This pressure forces the magma through the spinneret which delivers it to the exterior. The magma upon contact with the external water or with air or oxygen has its physical state again altered. The altered magma forms the "stinging" filament. The discharge and formation of the filament of magma ("stinging thread"), therefore, involves two conversions of physical states rather than the eversion of an inverted tube. The only eversion that is involved, in the discharge of the penetrant, is that of the spinneret and its shaft, which at the end of the discharge turns inside out.

58. The influence of $CaCl_2$, NaCl and osmotic pressure on the size of food vacuoles in Vorticella. William J. Bowen, University of North Carolina. (Lantern; 10 min.)

The Peritrich Vorticella (similis?) offers opportunity to study the factors which control the size of food vacuoles in the ciliates. Vorticellae can be mounted on a slide, and studied for hours with an oil immersion lens while the solution under the cover slip is being changed. The food vacuoles of Vorticella are spindle-shaped, having a short and a long axis. Under various conditions the former varies in length while the latter remains constant. Consequently, the length of the short axis serves as a criterion of the size of the vacuoles. An experiment consisted of measuring the width of several food vacuoles of one specimen in each of several concentrations of a salt, or lactose, when the vacuole left the gullet during about 25 min.

In a series of experiments on the effect of $CaCl_2$ the food vacuoles were an average of $30.0 \pm 3.9\%$ smaller in .0001 M than in redistilled water, $19.3 \pm 5.6\%$ smaller in .001 M, and $18.7 \pm 3.4\%$ smaller in .01 M.

In a similar series on the effect of NaCl the vacuoles were an average of $5.3 \pm 4.9\%$ smaller in .00014 M than in redistilled water, $23.7 \pm 3.4\%$ smaller in .0014 M, and $26.1 \pm 7.9\%$ smaller in .014 M.

These results show that $CaCl_2$ causes a greater decrease in vacuolar size than NaCl but do not show unequivocally whether the decrease is due to the cations or to the accompanying increased osmotic pressure. Other experiments show, however, that when osmotic pressure is increased, as by dissolving lactose in redistilled water, the vacuoles increase, rather than decrease, in size.

59. Concerning the directness of effect of factors which influence the size of food vacuoles in the ciliate Vorticella. William J. Bowen, University of North Carolina. (Lantern; 5 min.)

Dogiel and Issakowa ('27) report that Paramecium in a solution of $BaCl_2$ forms small, spindle-shaped food vacuoles but they do not indicate whether this effect is directly on the vacuolar membrane or indirectly through the cytoplasm. Data from experiments on the effects of $CaCl_2$, NaCl and lactose on the width of the normally spindle-shaped food vacuoles of Vorticella (similis?) have bearing on this problem.

A concentration of salt which causes reduction in vacuolar size will do so within 2 minutes, or less, after the solution is applied. In one experiment a vacuole formed while the specimen was in redistilled water was 15.6 μ wide. Then .001 M $CaCl_2$ was substituted for the redistilled water and in 30 seconds a vacuole only 10.2 μ wide was formed. In another instance a vacuole formed in .001 M $CaCl_2$ was 17.0 μ wide. Then .01 M $CaCl_2$ was applied and in 60 seconds a vacuole only 6.8 μ wide was formed. These results are supported by numerous instances.

Also, when .026 M lactose, which causes large vacuoles, was substituted for .014 M NaCl, which causes small vacuoles, the size of vacuoles formed within 2 minutes was 33% to 80% larger than the last ones formed in NaCl.

These results indicate clearly that factors which influence the size of food vacuoles in Vorticella act directly on the vacuolar membrane. Other data taken after time had been allowed for dispersal of the salt (especially $CaCl_2$) throughout the cytoplasm, indicate that such dispersal causes the vacuoles to increase in width.

60. Electron microscope studies of the structure of paramecium trichocysts. Marie A. Jakus, Cecil E. Hall and Francis O. Schmitt, Department of Biology and Biological Engineering, Massachusetts Institute of Technology. (Lantern; 15 min.)

Trichocysts were obtained by treating paramecia with reagents, by allowing them to dry on the electron microscope specimen grid, or by electrical stimulation (condenser discharges). High-speed photography shows that the trichocysts are expelled very rapidly after electrical stimulation. In the fresh and dry condition they show birefringence which is positive with respect to the long axis. In the ultramicroscope the pointed tip and the edges of the shaft of fresh trichocysts appear bright while the inner portion of the shaft is optically empty.

In the electron micrographs (EMG's) the tip is very dense, tapering gradually to a point. Tips which have become detached from the shaft are frequently observed, in which case both members retain their original shape. The striking characteristic of the shaft in its cross-striated appearance, resembling in this respect EMG's of collagen fibrils. In contrast to the latter is the tendency to appear herring-boned, the striations tapering to a point at the edges of the shaft. Moreover, extruded trichocysts show no longitudinal cleavage as do collagen fibrils. The distance between striations (spacing) is fairly uniform over the length of a given trichocyst and in trichocysts extruded from a single paramecium. Spacings from 500–800 Å have been observed, the greatest number occurring at 600–650 Å.

The unextruded trichocyst appears as an opaque, tapering rodlet from which the tip protrudes. In the explosive extrusion the shaft becomes about ten times the length of the unextruded form. The extremely regular structure noted above must play an important role in this process.

Cytology and Protozoology (Sect. C)

61. A POSSIBLE SOURCE OF NEW EVIDENCE ON THE DIFFERENCE BETWEEN MITOSIS AND MEIOSIS. C. A. Berger and Sr. Mary Grell, Fordham University. (Lantern; 15 min.)

Cells forming the epithelium of the larval ileum of mosquitoes (2 n equals 6) increase greatly in size, not in number, during larval life. This increase in size is accompanied by a corresponding increase in chromosome number through repeated chromosome reproduction in the resting nuclei which become highly polyploid (16 n, 32 n). During pupation these cells divide. In early prophase six groups of multiple sister chromonemata appear. The individual sister strands are distinct but are closely associated in one compact group during prophase contraction. This association is as close as synaptic pairing in meiosis and apparently differs from it only in the manner of origin, iliac cells showing no synapsis in the sense of coming together of threads previously separated. The groups of associated threads appear as groups in earliest prophase indicating that they had undergone reproduction without separation in the preceding resting stage. At late prophase each group falls apart into metaphase chromosomes consisting of two chromatids in paranemic coils. Homologous groups of sister strands show the paired association characteristic of the diptera. A detailed comparative study of these multiple complex prophases and of meiotic prophase is being made in the hope of obtaining decisive evidence on some of the assumptions inherent in current theories of the difference between mitosis and meiosis.

62. SOME EFFECTS OF CENTRIFUGAL FORCE UPON THE POLARITY OF GERMINATING POLLEN GRAINS. H. W. Beams and Norman Sulkin, State University of Iowa. (Lantern; 15 min.)

Control pollen grains from the snapdragon were germinated in a culture medium composed of 1% agar and 10% sugar having a pH of 8.8. At room temperature the pollen tubes were observed to appear from 40 to 80 minutes after being embedded or sown on the surface of the medium.

So far as is known pollen grains do not possess a predetermined polarity until near the time of the appearance of the pollen tubes. In the following experiments pollen grains were embedded in the agar-sugar medium in a glass cell and centrifuged in the dark at approximately 1500 times gravity until the pollen tubes appeared. Counts of a thousand cells that germinated in the centrifuge showed 66.7% coming out of the centrifugally directed pore, 20.3% at right angles to the centrifugal force and 13.0% directed centripetally.

When centrifuged under similar conditions in an ultracentrifuge at approximately 57,000 times gravity a count of 1,000 grains with tubes showed 90.03% coming out of the centrifugally directed pore, 8.2%, directed laterally and

1.77% directed centripetally. Ultracentrifuged pollen grains showed stratification of their intracellular materials. Because of the nature of the culture medium the grains were not free to rotate in the centrifuge. Therefore, the centrifugal force has affected directly or indirectly the polarity of these cells. Studies on the polarity of pollen grains are being continued.

63. The chromatophores of Rana pipiens. Hans Elias, David Cohen and Irving Lieberman. Middlesex University. (Opaque projector; 15 min.)

Five kinds of pigment cells determine the changing color of the grass frog: (1) epidermal melanophores, (2) xanthophores, (3) leukophores, (4) intracutaneous melanophores, (5) erythrophores.

The two kinds of melanophores and the leukophores are not essentially different from the corresponding cells of other Rana species. The xanthophores, however, are peculiar in as much as many of them remain in a state which in other Anurans and in certain Teleosts represents only a transitional stage of development, which occurs in Anurans during the period of metamorphosis. Such a flat cell contains scattered throughout the cytoplasm colorless stromata, and near the nucleus are accumulated drops of liquid lipochrome which form a xanthoma. While in other species the xanthomata become split up and are adsorbed into the stromata, this is not the case in all xanthophores of Rana pipiens.

The erythrophores contain large crystals or small granules of purplish-red lipochrome. Sometimes purplish-red crystals and large drops of yellow lipochrome occur in the same cell.

64. Nuclear phenomena in the mouse liver from birth to maturity. J. Walter Wilson, Brown University. (Lantern; 8 min.)

Nuclear phenomena were studied in the livers of a series of 86 dated mice from birth to 255 days. It is ordinarily said that mitosis ceases in mammal livers shortly after birth, and the problem has been to account for further growth of the liver and the production of binucleate cells. Mitotic figures were found in much older animals than was expected. The number of mitoses varies so irregularly that exact counts lack significance, e.g., of two animals 57 days old one had 80 mitotic figures in a section, the other 4. In general the number falls off with age and most older animals have none. Mitosis would account for some if not all of the growth.

A large proportion of prophases and telophases occurs e.g., at 57 days, the counts are 23 prophases, 4 metaphases, 8 anaphases, 34 telophases. The prophases and telophase may be unusually prolonged.

The occurrence of late anaphases with no cleavage furrow and of binucleate telophases favor the "cytoplasmic lag" theory of the origin of binucleate cells.

The occurrence of a large number of binucleate prophases and of cells with two clumps of chromosomes, as if from the breakdown of binucleate prophases, may indicate that such cells divide mitotically.

Multinucleate cells (4–8) were found in several older individuals. No clue to their formation was found.

It is suggested that cessation of mitosis in the mammalian liver after birth may be a function of time and not of relative age and maturity.

65. THE CYTOLOGY OF THE MOUSE LIVER IN A CONTROLLED DIURNAL CYCLE. Helen Wendler Deane, Brown University. (Introduced by J. Walter Wilson.) (Lantern; 7 minutes.)

Mice were trained to eat during 2 hours of the day only and were killed at 3-hour intervals following the beginning of feeding. A study of some of the cytological changes in their livers has been made. There is an initial rise in liver-cell size, followed by a small decrease at 9 hours, a secondary rise and then a gradual fall to the 24-hour level. These changes in cell size are associated with the following phenomena. Glycogen fills all of the cells within three hours and begins to decrease at about 15 hours, disappearing first in the central part of the lobule. Fat appears in the central-zone cells for a short time at about 15 hours. Granules of precipitated bile components become gradually more abundant during the 24-hour period. The mitochondria in Regaud preparations exhibit the following tendencies: in the central zone of the lobule; the fine filaments are at first tangled and clumped together but later become discrete and sparse. In the middle zone, the mitochondria are short, swollen and often clumped in the first 12 hours and then gradually become finer and more elongated. In the peripheral zone, the large mitochondria are initially filamentous but rapidly enspherulate, becoming slightly elongated only toward the end of the cycle.

The changes in cell content described here are not in precise agreement with those described by Forsgren and others in the uncontrolled diurnal cycle in various rodents. However, two different series gave consistent results.

66. DESTRUCTIVE CHANGES IN THE PANCREAS IN OLD AGE, SHOWN BY A STUDY OF WISTAR INSTITUTE RATS.[1] Warren Andrew, Baylor University College of Medicine. (Lantern; 15 min.)

Sections of pancreas from fifty normal rats, including both the Albino and the Gray Norway animals, have been studied to ascertain what changes, if any, might be seen at various ages. The organs in young and "middle-aged" animals present the typical histological picture; but beginning at about 700 days of age, a marked and very specific type of alteration makes its appearance.

The early changes are characterized by a proliferation of the cells of the interlobular ducts. New branches arise from these ducts and such branches, often without a distinct lumen, push their way into the lobules, between the individual alveoli.

In more advanced stages of the process, the masses of duct cells acquire each a definite lumen, forming intralobular ducts, a feature never present in the pancreas in younger animals. The ducts undergo a process of expansion until finally they appear as cystic spaces lined with a simple squamous epithelium. They are at times completely empty, at other times partially or wholly filled with cellular debris.

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[1] This work is part of a general study on aging organized by Dr. E. J. Farris of The Wistar Institute of Anatomy and Biology with the aid of cooperating scientists and with the support of the Samuel S. Fels Fund. The author wishes also to acknowledge support of his individual project by a grant from the Ella Sachs Plotz Foundation.

The extreme thinning of the walls of such spaces leads eventually to their rupture, with the formation of larger cavities. An entire lobule may be thus converted into a series of cyst-like cavities. Pressure of the expanding spaces is accompanied by an atrophy and disappearance of secreting alveoli and islets of Langerhans.

The process is present in all of the senile animals studied but varies considerably in the number of lobules involved.

67. The regeneration of Eisenia foetida (Sav.) as determined by the lympho-trophic system. Emil Liebmann, Department of Zoology, Duke University. (Introduced by L. H. Hyman.) (Lantern; 15 min.)

Eleocytes — a trophic type of lymphocyte — are essential in regeneration, which does not occur if their number is too small. They are produced by the chloragogue and supply the wound and regenerate with nutritive and activating substances; they are not identical with neoblasts.

In regeneration they function as regularly shaped aggregates resembling a tissue. There are two varieties of these aggregates: one instrumental in head, the other in tail regeneration. Their cells show differences in structure and content indicating different chemical composition, which is apparently responsible for the specificity of head and tail regeneration.

The aggregates instrumental in head regeneration are found anteriorly of the gut. Their location and distribution determine the gradient and limit of head regeneration.

Aggregates inducing tail reconstitution are formed only during regeneration. The gradient and limits in tail regeneration are determined by the quantitative differential of the chloragogue and its extent. Failure of isolated terminal pieces to regenerate is attributed to their lack of chloragogue.

Both aggregates are structurally and functionally polarized, which determines the different forms of polarity in head and tail regeneration. Once formed, they are not affected by exogenous factors like the individual eleocyte or the chloragogue. The mutual independence of head and tail regeneration and their different response to exogenous changes is explained by the different ways their aggregates are formed.

The lympho-trophic system is apparently the sole determinant in regeneration; it is tranportative and not transmissive in nature. The nervous system seemingly plays no role in regeneration.

68. Imbedding without dehydration. Robert T. Hance, Duquesne University. (10 min.)

Tissues may, without previous dehydration, be infiltrated with a newly developed material that gives adequate support for serial sectioning. The entire process of preparing a slide, from the supporting medium to the final cover glass cement, is carried out with water soluble materials. This prevents the loss of those cell constituents that are soluble in the various commonly used dehydrating fluids.

69. The conjugation of Vorticella microstoma. Harold E. Finley, Morehouse College. (Lantern; 15 min.)

In Vorticella microstoma conjugation occurs when the microconjugant fuses with and enters a larger attached individual, the macroconjugant. The macroconjugant and microconjugant originate from a vegetative individual as the result of an equal division of the micronucleus accompanied by an unequal division of the macronucleus and endoplasmic components. The entire conjugation period may be arbitrarily divided into six intervals: (1) firm attachment and entering, 30 minutes; (2) progamic nuclear divisions and shedding the microconjugant's pellicle, 30 minutes; (3) synkaryon formation, 5 to 15 minutes; (4) metagamic nuclear divisions and first reorganization fission, 12 to 18 hours; (5) second and third reorganization fissions; 2 hours; (6) growth of the new macronucleus and resorption of the remaining old macronuclear fragments, 2 hours.

Each conjugant produces one spindle-shaped functional pronucleus. These fuse and form the synkaryon which gives rise to the metagamic nuclei. All progamic divisions are mitotic divisions. The diploid number of chromosomes is four. The microconjugant's micronucleus undergoes three progamic divisions. The macroconjugant's micronucleus undergoes two progamic divisions. In each conjugant, the last progamic division reduces the chromosome complement to the haploid number. Since the microconjugant's first progamic micronuclear division occurs before it enters the macroconjugant it has been considered a preliminary division (Maupas, 1888).

Three metagamic nuclear divisions result in the formation of eight nuclei of which one becomes a micronucleus and seven form macronuclear anlagen. Three reorganization fissions distribute the seven macronuclear anlagen to seven individuals and the micronucleus divides at each fission.

70. Double animals and multiple simultaneous mating in variety 4 of Paramecium aurelia in relation to mating types. T. M. Sonneborn, Indiana University. (Lantern; 15 min.)

In variety 4 of Paramecium aurelia pairs that have conjugated occasionally fail to break apart, but remain connected by their paroral cones. From their separated anterior and posterior regions normal single animals arise at fission; the intervening region of union either develops into a non-viable multiple monster or (in 3 different instances) into an entirely double individual. The latter have produced clones of "parabiotic twins" still thriving after 3 months. In all 3 cases, the single animals produced from the separated regions of the two united exconjugants yielded clones agreeing in mating type with each other and with the subsequently developed double animals. As the two mates were of different mating types at the start of conjugation, one of each pair must have changed its type. This is remarkable because mating type rarely changes at conjugation in this variety. When double animals are mixed with single animals of the other mating type, all the resulting conjugations are of double animals with single animals, showing that conjugation takes place only between animals of different mating types. In these mixtures the double animals conjugate simultaneously

with two single animals, one on each side. When two cultures of double animals differing in mating type are mixed, chains of up to 9 animals attempt to conjugate simultaneously, for each double animal can receive 2 double mates of the other mating type, 1 on each side.

71. The effect of Trichomonas vaginalis on tissue culture cells. M. J. Hogue, Medical School, University of Pennsylvania. (Lantern; 15 min.)

Human tissue cultures were made from foetuses 3–5 months old. We obtained good growths of fibroblasts from pieces of intestine, lung and leg muscle. These tissues were grown in a roller tube apparatus and transferred to hanging drop cultures when needed for experiments. Chick tissue cultures were made from 8–9 day old chick embryos. Most of the cultures were made from the intestine. They had good growths of epithelium, mesothelium, fibroblasts and sympathetic nerves. Dr. Trussell's bacteria-free strain of T. vaginalis was used. Controls were run for all experiments.

When rich cultures of T. vaginalis were added to a tissue culture, the tissue cells drew in their fine processes, became granular and the nuclear membrane and nucleolus were very distinct. Small vacuoles appeared. Most of the cells were dead in 4–5 hours, though the trichomonas lived on for several days. When the filtrates of old cultures were added to tissue cultures the cells went through the same degenerative changes. After cultures had been heated at 56° for 15 and 30 minutes and at 60° for 20 minutes they still caused the death of tissue culture cells, though the process was slower and there was occasionally some recovery.

Metts tubes and Gillman and Cowgill's test for proteolytic enzymes gave negative results.

T. vaginalis gave off a substance which was toxic for the tissue culture cells.

Motion Pictures

105. Social organization and leadership among sheep. J. P. Scott. Wabash College. (Kodachrome.)

The film shows the daily and seasonal cycle of social life among domestic sheep, with additional studies of wild Bighorn rams. In descending order of importance, social behavior may be classified as allelomimetic (mutual imitation), epimeletic (care of others), fighting, sexual and shelter-seeking. For males sexual and fighting behavior are more important and epimeletic non-existent. For lambs nursing and et-epimeletic behavior (calling or signalling for attention and care) are of great importance. These types of behavior result in the formation of several groups: (1) rams, (2) lambs, (3) ewes and lambs, (4) ewes, rams and lambs. Social dominance or leadership may be developed through any type of social behavior. The factors affecting leadership may be divided into innate causes, the most important of which are hormones and heredity; and environmental factors, the most important of which are learning and familiarity with the environment. In the case of learning, dominance may be achieved by association with either an injury or a reward. In sheep the most important type of dominance is achieved through a reward — association of the mother with nursing. The result

is that the elder ewes become the leaders of the flock in most activities. In a ram flock, animals born the same year tend to stick together. When the rams of two years are mixed the older rams often take the lead. Some evidence is presented to show that the situation in mountain sheep is similar. Comparative studies of this nature should form a useful background for human sociology.

106. THIAMIN (VITAMIN B_1) DEFICIENCY IN THE CAT. Guy M. Everett and Dietrich C. Smith, University of Maryland School of Medicine.

A 16 mm. motion picture film was made showing the behavior of two cats during the development of thiamin deficiency. After 3 weeks on the vitamin B_1 deficient diet, during which time the two cats lost approximately 20% of their body weight, the animals showed muscular weakness and marked disturbances in postural tone. Walking was awkward and unsteady. The righting reactions were much impaired and pupillary constriction in bright light was lessened. Interest in food had ceased. At this time any slight stimulus may have produced a convulsive seizure lasting several minutes. Both clonic and spastic spasms occurred.

Upon the injection of 1 mg. (333 units) of thiamin chloride the animals showed within an hour improved muscular coordination and a return of appetite. Convulsive seizures could no longer be evoked. In 24 hours recovery was practically complete.

107. THE BIOLOGY OF REPRODUCTION IN RATS.[1] Edmond J. Farris, The Wistar Institute of Anatomy and Biology. (Kodachrome.)

The kodachrome film was planned to demonstrate various reproductive processes in the albino rat, emphasizing new findings, namely, the release of the spermatozoa from the uterus to the oviduct and ovary at about ovulation time, and four stages in mating behavior of male and female during her estrous cycle.

The film demonstrates as well the technique to establish accurately puberty, estrous cycles, and menopause by running activity, the physiology of reproduction, and parturition.

[1] This investigation was aided in part by a grant from the Samuel S. Fels Fund.

108. FURTHER OBSERVATIONS ON THE SEXUAL BEHAVIOR OF HORMONALLY TREATED BROWN LEGHORN FOWL. L. V. Domm, David E. Davis and Ben Blivaiss, Whitman Laboratory of Experimental Zoology, The University of Chicago.

To interpret earlier observations on sexual behavior of hormonally treated fowl new experiments were undertaken on capons receiving estrogen and androgen and on pullets receiving only androgen.

Three adult capons receiving daily injections of 0.33 mg. alpha-estradiol plus 1.00 mg. testosterone waltzed, circled, and copulated on third day and crowed on thirteenth, sixteenth and seventeenth days respectively. In three capons receiving 1.00 mg. stilbestrol plus 1.00 mg. testosterone daily, one waltzed, circled, and copulated on fourth and crowed on fifth day; a second copulated on sixth and

crowed on eighth day, while third circled on tenth, copulated on fourteenth, and crowed on twenty-second day of injections.

Three pullets (112 days old) received 0.50 mg. testosterone daily. One crowed on fifty-second day and showed circling and waltzing on seventy-fourth. The second crowed on eighty-eighth day but never showed particular interest in either sex, while third showed no response even after 109 days. In three pullets (112 days old) receiving 1.00 mg. testosterone, one crowed on fortieth, the other two on forty-third day of injections. One waltzed and circled on fifty-eighth, the second on sixtieth, and third on one hundred and sixth day. In a third series of three pullets (138 days old) receiving 1.50 mg. testosterone, one crowed on seventeenth, the other two on eighteenth day. Circling and waltzing were observed on thirty-second, forty-fifth and one hundred and eighteenth days respectively.

Of particular interest is the fact that capons receiving mixed injections developed essentially normal masculine behavior, whereas intersexual males usually show a strikingly modified behavior pattern. The absence of complete copulatory pattern in treated pullets agrees with earlier observations on similarly treated poulards.

109. The sexual behavior of Rana pipiens and its modification by brain injury in the male. Lester R. Aronson and G. Kingsley Noble, Department of Animal Behavior, the American Museum of Natural History. (Introduced by William Etkin.)

The sexual behavior of the leopard frog, Rana pipiens, induced in the laboratory following anterior pituitary injections, was investigated. The phases studied included: (1) the sex call of the male; (2) the warning croak of the male and female; (3) post-amplexus recognition by the male of an ovulated female as distinguished from an unovulated female; (4) the pattern of oviposition; (5) pseudo-oviposition (oviposition movements without the deposition of eggs) induced in unovulated females following intraperitoneal injections of physiological saline solution; and (6) the release behavior of the male following oviposition. This behavior will be illustrated by motion pictures.

Removal of the entire forebrain, diencephalon, tectum, cerebellum, and anterior edge of the tegmentum did not affect the ejaculatory responses of the male, but invasion of the posterior tegmentum resulted in the complete loss of these reflexes. The sex and warning croaks disappeared following lesions in the inferior colliculi.

Following the removal of the entire forebrain including the pre-optic area (telecephalon medium) males no longer pursued and attempted to clasp other frogs, but if placed on a female, such animals showed normal amplexus and oviposition. Males with this operation often failed to release the female at the termination of oviposition. Small lesions affecting the preoptic nuclei usually abolished the release response, but not the male's pursuit of other frogs. Removal of the entire forebrain excepting the preoptic area and various other smaller forebrain lesions caused no observable changes in the mating pattern.

110. Return of vision four times in the same adult salamander eye repeatedly transplanted. L. S. Stone, Anatomical Laboratory, Yale University School of Medicine. (Introduced by W. O. Nelson.)

A colored motion picture shows the operations involved in transplanting the entire eye of the adult salamander (Triturus viridescens) from one host to another. Within 3 months after operation degeneration of the retina, optic nerve and lens is followed by regeneration each time the eye is grafted. At the end of this period the transplant becomes again a normal appearing functioning organ. The living eye is shown at various stages after operation and proof of return of vision as well as other functions, is demonstrated in the same eye on each of its four hosts.

111. Cine-photomicrographs of certain aspects of the motility of human spermatozoa. John MacLeod, Department of Anatomy, Cornell University Medical College. (Introduced by L. V. Domm.)

The film demonstrates the loss of motility which takes place when human spermatozoa are deprived of glucose and the effect of added glucose in restoring motility. The return of motile activity is shown in individual spermatozoa under high magnification.

112. Functional activity of the blood capillaries in the rat. C. G. Grand, Department of Biology, Washington Square College, New York. (Introduced by Robert Chambers.)

The scenes are of the circulation in the mesoappendix showing the topography of a typical capillary bed and responses of the various components under normal and experimental conditions.

113. Fixed persistence in the rat of spinal reflex patterns rendered extremely maladaptive by cross union of sensory nerves. R. W. Sperry, Harvard Biological Laboratories. (Introduced by A. B. Dawson.)

After crossing nerves from the left hind foot to the right hind foot in 16 rats, the right foot became reinnervated only by nerves that originally supplied the left foot. This resulted in all cases in a false localization of sensation and a reversal of hind limb reflexes mediated by the crossed nerves. Although extremely maladaptive, the reversed spinal reflexes persisted through prolonged training over a period of ten months without correction or inhibition by central nervous adjustment.

114. Effects of colchicine experiments on chicken. Edna Higbee, University of Pittsburgh.

The moving pictures show the development of combs and wattles of chicken when 0.02 cc. of a 0.0001% colchicine solution was injected into embryos of 72 hours and 13 days of incubation, and into chicks 1 and 2 days old. The film shows the chickens at various stages of their development. The oldest chicken is now 3 years and 4 months old.

Cellular Physiology

115. Characteristics of human sympathetic ganglion cells cultivated in vitro. M. R. Murray and A. P. Stout, College of Physicians and Surgeons, New York.

See Abstract No. 85.

Cytology

116. Water soluble micrological materials. Robert T. Hance, Duquesne University. (Demonstration.)

The methods of using the new all water technic for making serial sections will be demonstrated.

Embryology

117. An hereditary syndrome of abnormalities of spine, urogenital system and intestine in mice. S. Gluecksohn-Schoenheimer and L. C. Dunn, Columbia University.

Monsters with a certain syndrome of malformations in the posterior trunk region may appear in mice of different genotypes. The syndrome consists of a combination of several of the following: shortening of the spine and spina bifida; complete absence or size reduction of the kidneys, ureters and bladder; abnormalities of the genital ducts; atresia of colon, rectum and anus, and presence of a cloaca. This syndrome may appear in mice: (1) as an effect of a dominant gene *Sd*, (2) as an effect of a recessive gene *u*, and (3) due to the interaction of 3 tail mutations at and near the *T*-locus.

A fourth group comprises occasional sporadic cases among mice carrying at least two different tail mutations.

This material indicates a close developmental interdependence of the different organ systems of the posterior trunk region. The syndrome seems to be a response which is evoked by hereditary changes at several different loci.

General Physiology

118. The discharge of the penetrant (a nematocyst) by hydra. William A. Kepner and Herbert Taylor, Miller School of Biology, University of Virginia.

Demonstrations will be made of the discharge of the nematocysts into air. It will be shown that the penetrant's discharge is inhibited in an atmospere of CO_2. The demonstration will indicate that the penetrant is not a sac that, upon discharge, everts a tubular filament; but a spinneret which, upon discharge, spins a silk-like thread of magma.

119. Asterias stomach tissue in anesthesia. Vera Koehring, Hunter College of the City of New York. (Introduced by Earl A. Dennis.)

With the starfish Asterias anesthesia may be induced so rapidly that when the star is placed oral side up in sea-water containing chloroform, ether, $MgSo_4$ or

HCl, the turning reaction cannot be completed. As anesthesia progresses the stomach is everted, at first as an entirely transparent membrane. With complete anesthesia the stomach tissues become opaque, not only generally, but markedly more opaque in a ramification of densely white areas in the several lobes.

This macroscopic change from transparency to opaqueness is a phenomenon of anesthesia and does not occur in the stomachs of controls during the normal feeding process. The change is readily and completely reversible upon recovery from anesthesia. When the star is placed in fresh sea-water the opaque tissues regain transparency and are withdrawn into the body while turning and locomotion activities are resumed.

The material thus demonstrates somewhat extensive membranes in which macroscopically reversible coagulation phenomena occur during anesthesia.

Morphology

120. Stereobalanus canadensis (Spengel), a little-known enteropneustan from the coast of Maine. Edward G. Reinhard, Catholic University of America.

In 1940 and 1941, dredging parties of the University of Maine Marine Laboratory collected complete living specimens of Stereobalanus canadensis at the mouth of Sullivan's River in Frenchmans Bay. Known previously only from four fragments described (1893) by Spengel from Nova Scotia waters, this enteropneustan presents morphological peculiarities of great interest. Preserved specimens, slides, and color sketches of the animal will be exhibited.

Protozoology

121. Demonstration of the cytosomic morphology of Paramecium polycaryum. E. M. Opton, Yale University. (Introduced by L. L. Woodruff.)

A study of the morphology of P. polycaryum provided the opportunity to compare in detail a member of the "bursaria group" with the accounts and figures of members of the "aurelia group" of the genus Paramecium. Ectoplasmic differentiation has been studied by the application of the wet methods of Gelei, which have received limited use in this country. The components of the silverline system are selectively demonstrated by aniline dyes and Bodian impregnation, which also reveals the trichocysts.

P. polycaryum shows lability in the patterns assumed by the supporting lattice. The relation of trichocysts to the transverse fibers of the lattice is expressed both by trichocyst granules and trichocyst apertures. The granular complex at the center of the lattice polygons is comprised of an accessory granule, a ciliary granule, and a secondary granule, arranged as the points of a triangle. The ciliary granule and the secondary granule lie along the neuroneme which connects these with their counterparts in other polygons of a meridional row. The accessory granule is at the apex of the triangle, and is to the left of the neuroneme.

In contrast to the description of the penniculus in species belonging to the "aurelia group," in which the number of ciliary rows is 8, the penniculus of P. polycaryum is provided with fifteen rows.

Endocrinology (Sect. A)

72. SUPPRESSION OF METAMORPHOSIS IN THE GRASSHOPPER MELANOPLUS DIFFERENTIALIS. Isabelle Weed Pfeiffer, Osborn Zoological Laboratory, Yale University. (Lantern; 15 min.)

Of 15 nymphs which, during the early part of the fifth stage, received grafts of 2 to 6 corpora allata from young adult females while retaining their own corpora allata, 12 failed to undergo metamorphosis at the end of the sixth stage. Instead, although of adult size, they remained nymphal in character of exoskeleton; in pigmentation pattern, particularly on the head and pronotum; and in having a rounded pronotum with clearly demarcated ecdysial line, instead of the squared pronotum with mid-dorsal ridge of the adult. Their wings varied from the wing-bud type to the dry wing of the adult. Six of these animals underwent one supernumerary molt before they died or were sacrificed, and one underwent two, thus attaining considerably greater than adult size. The first observable response to the grafted corpora allata was the assumption of a green color during the sixth stage because the blood, visible through unpigmented areas of the exoskeleton, had become green. Normal nymphs had yellow blood. Eight of the nymphs receiving corpus allatum grafts were, in the same operation, castrated and supplied with pieces of adult ovary; 7 retained their ovaries and received only corpora allata. This difference in treatment did not affect the results.

Presumably the adult corpora allata supplied the nymphal system with sufficient "metamorphosis inhibiting" or "nymphal" hormone, such as demonstrated by Wigglesworth, to suppress metamorphosis. Whether the effect on blood color was part of the same reaction is not clear.

73. RESPIRATORY RESPONSES OF NORMAL AND CASTRATE GOLDFISH TO TELEOST AND MAMMALIAN HORMONES. Arthur D. Hasler and Roland K. Meyer, University of Wisconsin. (Lantern; 15 min.)

When goldfish are injected with carp pituitary preparation spawning was induced prematurely; moreover, the oxygen consumption was increased as much as 76% (mean of this group was 36%). This rise in oxygen consumption did not occur in castrate goldfish similarly treated. It was concluded, therefore, that the oxygen consumption accelerating effect observed in normal fish was the result of the stimulation of the gonad and not of the tissues in general. The luteinizing fraction of sheep pituitary did not increase the oxygen uptake nor did it induce spawning. The negative results of previous workers with thyroxine on fish are corroborated. In the study of fish metabolism the dropping mercury electrode method for the determination of dissolved oxygen was found more applicable than the Winkler method.

74. THE EFFECT OF "HIGH ALTITUDE" UPON THE PITUITARY AND ADRENAL OF THE WHITE RAT. Gerard R. Pomerat and Steven M. Horvath, Harvard University. (Lantern; 15 min.)

Rats kept in a chamber under reduced pressure corresponding to that obtained at an altitude of 25,000 feet undergo a 15 to 20% weight loss within 2 days. The adrenals of animals kept in the chamber for 48 hours show a marked reduction

in the amount of osmiophilic material present in most cells of the cortex, although numerous granules can still be seen in the zona glomerulosa. After 84 hours under reduced pressure the adrenal cortex is densely packed with osmiophilic granules distributed throughout the zona glomerulosa and fasciculata. These granules are quite regular in size and shape. The zona reticularis also contains much osmiophilic material, but the masses are irregular in shape, size and distribution. No further change can be seen in the cells of the cortex when the experiment is continued for 168 hours.

The pituitary glands of rats in the 48-hour experiment appear normal although many basophiles show densely-stained granules and some show small colloid-filled vacuoles. After 84 hours the pituitaries show a slight reduction in the number of acidophiles and a marked increase in basophiles. The latter frequently show a strong affinity for the blue dye and many contain colloid-filled vacuoles. The pituitaries of rats kept in the chamber for 168 hours show no marked deviation from the normal. These changes in the pituitary appear to bear some relation to those taking place in the adrenal cortex.

75. The anterior pituitary of the broody fowl. F. Payne, Indiana University. (10 min.)

The changes which occur in the anterior pituitary of the fowl during the period of broody behavior are: 1. Nuclei of nearly all basophiles stain darker than in actively functional basophiles and the cytoplasm becomes reduced. 2. The nuclei of some of the granular acidophiles (A_1 type) also become darker. 3. In a region approximately in the middle of the gland dorso-ventrally and about one-third the distance from the anterior end, a series of changes takes place in which all cell types are involved. 4. The acidophiles and basophiles whether previously modified or not, become reduced in size due primarily to change in the volume of cytoplasm. 5. The nuclei of the acidophiles become clear and vesicular and the cytoplasm forms acidophilic granules. 6. These small acidophiles become the dominant type of cell. 7. The nuclei of the basophiles remain dark until late in the transition, but later become clear. With present information one cannot say the cytoplasm ever contains acidophilic granules. 8. The acidophiles of the A_2 type also become reduced in size but they can be distinguished by their darker cytoplasm and nucleoplasm. 9. The chromophobes may change into the small modified acidophiles. 10. The region of these modifications expands in all directions until nearly the whole of the gland is involved. 11. Near the end of the broody period, the cells return to their former appearance and functions. 12. As injections of prolactin bring on broody behavior, the suggestion is made that these small acidophilic cells secrete prolactin.

76. Ovarian cysts in immature female cats following treatment with equine gonadotropin. William F. Starkey and James H. Leathem, Allegheny General Hospital and Rutgers University. (Lantern; 15 min.)

The response of 36 immature female cats to equine gonadotropin was studied. In many cases, excessive ovarian stimulation with cystic formation were noted. The initial effect of a single subcutaneous injection of 200 rat units (Cole-Saunders) of pregnant mare serum, Gonadin, is the production of large multiocular

cysts containing clear fluid and lined with granulosa cells. The corresponding uterine endometrium is in a proliferative state. Similar cysts have been noted to regress or to persist as simple retention cysts, which are lined by cuboidal epithelium, or to form corpus luetum cysts which are lined by luteal cells. That these luteal cells are secretory is shown by the production of a progestational endometrium. At 30 days after hormone administration, cysts in various stages of atresis have been noted.

77. PRECOCIOUS DEVELOPMENT OF SECONDARY SEX CHARACTERS IN JUVENILE BUFO FOWLERI BY INJECTIONS OF VARIOUS HORMONES. Albert P. Blair, Laboratory of Experimental Biology, American Museum of Natural History. (Lantern; 15 min.)

Male Bufo fowleri show secondary sex characters at a body length of 40–50 mm., females show no external secondary sex characters. Seventy juvenile B. fowleri, body length 33–49 mm., sex unknown, were divided into 7 groups of 10 each. One group received no injections. The remaining groups were injected subcutaneously at 3-day intervals for 30 days. Of 10 animals injected with chorionic gonadotropin, 5 developed male sex characters; autopsy showed these to be males and the other 5 females. One female of this group showed enlarged oviducts. Of a group injected with fresh whole female B. fowleri pituitary gland, 5 developed male sex characters; autopsy showed these to be males and the remainder 4 females and 1 male. One female of this group showed enlarged oviducts. Ten animals were injected with testosterone propionate; 2 died early in the experiment. The remaining 8 developed male sex characters; autopsy showed 3 females and 5 males. All showed enlarged oviducts. Of 10 animals injected with testosterone propionate plus fresh B. fowleri pituitary gland, 2 died early in the experiment. The remaining 8 developed male sex characters; autopsy showed 2 males and 6 females. All females and 1 male showed enlarged oviducts. Of a group injected with prolactin, 1 male developed male sex characters; oviducts were not enlarged. Of 10 animals injected with α-estradiol benzoate, none showed male sex characters; all showed enlarged oviducts. One male control developed male sex characters. None of the injections affected the clasping reflex.

78. FURTHER OBSERVATIONS ON THE EFFECT OF SEX HORMONES ON ERYTHROCYTE NUMBER IN THE BROWN LEGHORN. Elsie Taber and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago. (Lantern; 12 min.)

It was previously reported that injections of testerone propionate raised the erythrocyte number in capons and sinistrally and bilaterally ovariectomized poulards, while injections of various estrogens lowered the number in males, capons, and poulards. It was also shown that intersexual males, feminized by estrogen injection during incubation, had red cell counts characteristic of the female level.

Recent observations have shown that 15 daily injections of 0.5, 1.0, or 1.5 mg. of testosterone propionate into 9 pullets were sufficient to raise the red cell count 24.2%. Injections were continued, and counts made at 25, 35, 50, and 65 days showed 19.6, 20.8, 29.1, and 50% increases, respectively, over the original erythrocyte number. There appeared no correlation between dosage and degree of increase.

In an attempt to duplicate the hormone condition found in intersexual males mixed injections of 1 mg. testosterone propionate and .33 mg. alpha-estradiol benzoate were given 3 capons. The average increase after 25 daily injections was 25%, while 3 controls injected with 1 mg. testosterone showed a 50.5% increase and 3 controls injected with .33 mg. estradiol showed a 16.8% decrease. In all cases the counts were approximately normal 21 days after cessation of injections.

Inconsistent results were obtained as a result of mixed injections of 1 mg. testosterone and 1 mg. diethylstilbestrol in 3 capons. One showed an increase of 31%, another a decrease of 10.3%, and a third a decrease of 13.6% in erythrocytes after 25 daily injections.

These results seem to suggest an antagonistic effect of androgen and estrogen on erythropoiesis.

79. Factors modifying the response of thyroidectomized rats to low temperature. C. P. Leblond and J. Gross, Department of Anatomy, McGill University. (Introduced by R. Grant.) (Lantern; 15 min.)

Adult rats thyroparathyroidectomized for over 5 days previously die within a few days at 0°, while normal, parathyroidectomized and thyroxine-treated thyroidectomized survive for several weeks. These results confirm the importance of thyroid hormone in the response to cold.

The resistance of thyroidectomized animals to cold can be increased in 2 ways: (1) if the thyroid was removed only 12 hours before exposure to cold, a prolonged survival (over 10 days in females) was observed. Apparently the thyroid hormone which is known to remain in the body for several days after thyroidectomy facilitated the adaptation to cold; (2) if the thyroidectomized animals were maintained at various temperatures for several days before exposure to cold, the length of survival varied. Thus, thyroidectomized female rats pretreated at 32° died overnight at 0° while pretreatment at 22° resulted in an average survival of 6 days. This result, as also the increased metabolism revealed by a rise in food consumption to 180% of normal in the thyroidectomized rats surviving at 0°, showed that a marked adaptation to cold may be obtained in the absence of the thyroid.

80. Oxygen consumption of skin and hair growth after adrenalectomy in the white rat. Earl O. Butcher, Hamilton College. (Lantern; 15 min.)

Previous experiments have shown that an increase in oxidative processes in the skin favors the growth of the hair in the rat. Adrenalectomy has also been found to induce activity in resting follicles. Experiments have now been conducted to see if the hair growth following adrenalectomy is related to an increase in the oxygen consumption of the skin.

Determinations were made with a Fenn respirometer and in Ringer's-phosphate solution at 37.5°C. Skin of animals containing inactive hair follicles used about .9 cmm. of oxygen per mg. of dry tissue per hour. Forty-eight hours after adrenalectomy, the oxygen consumption had increased 14%. Sixty-six hours after adrenalectomy, and prior to the beginning of growth in the hair follicle the consumption reached 1.25 cmm. (39% increase) per mg.

Increased oxygen consumption of the skin results from adrenalectomy in the white rat, and these increased oxidative processes appear to induce hair growth in inactive follicles.

81. Effects of adrenal cortical hormones alone and in combination with prolactin on body and visceral weights in hypophysectomized pigeons. Richard A. Miller and Oscar Riddle. Carnegie Institution, Department of Genetics. (Lantern; 15 min.)

This is an investigation of the ability of hormones of the adrenal cortex, alone and in combination with a small quantity of prolactin, to prevent the loss of weight in body and viscera which follows the removal of the anterior pituitary gland. Groups of White Carneau pigeons 1.9 months old were thus hypophysectomized, injected for 10 successive days and then killed. Intake of food and water was measured.

Adrenal cortex extract (Wilson), and particularly desoxycorticosterone acetate (DCA), is able partially to maintain the weight of hypophysectomized pigeons. Also, the effect of DCA is additive to the effect of one unit of prolactin which alone is insufficient fully to maintain the weight of hypophysectomized pigeons. The various levels of daily dosage, and the final body weights expressed in percentage of preoperative weight, are as follows: (a) untreated hypophysectomized control, 78%; (b) adrenal cortex extract, 0.1 cc. 86%, 0.2 cc. 87%; (c) DCA, implanted pellet 84%, 1 mg. 90%, 1 mg. plus pellet 91%, 2 mg. plus pellet 94%, 3 mg. 94%, 5 mg. plus pellet 86%; (d) prolactin, 1 unit 92%; (e) 1 unit prolactin plus 1 mg. DCA, 101%.

Cortical hormones alone can maintain the weights of intestines and livers (effect with prolactin is additive) and markedly support the pancreas, but they have no similar action on thyroids, testes and adrenals. Food consumption is partially supported and water intake is much increased. Determinations of the water content of breast muscles and livers of some groups showed no significant hydration of these tissues.

82. The partial inactivation of desoxycorticosterone by the liver. Wilburn J. Eversole and Robert Gaunt, Department of Biology, Washington Square College of Arts and Science, New York University. (Lantern; 12 min.)

A total of 226 male rats of 50–60 gm. were adrenalectomized. Sixty served as controls and the remainder received desoxycorticosterone acetate (DCA) either by mouth, parenterally, or in pellet form.

One to three DCA-cholesterol pellets, each containing an average of 0.75 mg. DCA, were implanted at the time of adrenalectomy at, (1) various sites with hepatic portal drainage (e.g., spleen, mesentery), (2) sites with systemic drainage (e.g., subcutaneous tissues, kidney). The 65 animals of the former group had an average weight gain of 1.5 gm. per day and 45% survived the 30-day observation period. The 55 animals of the latter group had an average weight gain of 2.5 gm. per day and 67% survived 30 days. The weight change in control animals was negligible and only 5% survived.

In three other groups adrenalectomized rats received 0.2 mg. DCA daily for ten days either, (1) subcutaneously, (2) mixed with their dry food, or, (3) by gavage. The latter group was not helped by the hormone even in 0.5 mg. daily doses; and although the second group showed good survival the growth rate was about one-half that of the animals receiving subcutaneous injections.

It would seem, therefore, that the effectiveness of DCA in maintaining adrenalectomized rats is somewhat reduced when the hormone passes through the hepatic portal system, probably indicating partial inactivation by the liver.

Cellular Physiology (Sect. B)

83. THE EFFECT OF OXALIC ACID, SODIUM OXALATE AND OTHER CALCIUM PRECIPITATING AGENTS ON WOUND HEALING. Louis-Paul Dugal and Henri Langier. University of Montreal. (Lantern; 15 min.)

1. Due to purely hypothetical considerations, we have chosen calcium precipitating agents: oxalic acid, sodium oxalate, potassium oxalate and sodium fluoride, as being probably effective in speeding wound healing. Preliminary experiments have shown clearly that wounds treated with those substances heal much faster than those treated with Chloramine-T. All the results expressed in this paper always come from comparisons made on two approximately equal (measured in every case) wounds of the same animal treated differently: this precaution eliminates differences in healing time due to different individuals.

2. The effects of different concentrations of the four chemical substances mentioned have been studied, and drove us to the conclusion that the most effective solutions are the least concentrated (approximately 0.01 N).

3. The effects of one of those substances, oxalic acid 2%, have been studied in details: 19 experiments out of 23 have proven that wounds treated with oxalic acid 2%, dissolved in saline 8% heal much faster than control wounds treated with saline 8% only; this last concentration of saline has been adopted after a series of experiments in which different concentrations of NaCl solutions have been tested for their effects on wound healing: the 8% concentration is certainly as good as any other, and better than most. The time of healing of wounds treated with oxalic acid was reduced of 42% as a maximum and of 22% as an average.

4. The oxalic is effective during the whole period of cicatrisation.

84. FORMULATION OF ENZYME INHIBITIONS: SULFANILAMIDE, URETHANE, TEMPERATURE, PRESSURE. Frank H. Johnson, Henry Eyring, and R. W. Williams. Princeton University. (Lantern; 15 min.)

Three types of reversible combination of inhibitors with enzymes have been theoretically formulated, on the basis of a normally present, reversible thermal "denaturation" equilibrium (K_1) of the catalyst. The relation of change in over-all velocity of the enzyme action, to concentration of added inhibitor, to temperature, and to pressure, in each of the 3 types, is given by the following expressions, in which I_1 is the uninhibited rate, I_2 the inhibited, ΔE the energy of reaction, ΔV the volume change of reaction, ΔS entropy, X the molar concentration, and r the number of molecules of inhibitor combining with one molecule of enzyme: Type I, combination of inhibitor with a group not affected in "denaturation," i.e., indiscriminately with active and "denatured" forms of the catalyst, $\log_{10}\left(\frac{I_1}{I_2}-1\right) = -\frac{\Delta E}{2.303\,RT} - \frac{p\Delta V}{2.303\,RT} + \frac{\Delta S}{2.303\,R} + r\log_{10}X$.

In Type II, the inhibitor combines only with the "denatured," and in Type III only with the active form of the catalyst. The left-hand side of the equation is then replaced by $\log_{10}\,(I_1/I_2-1)\,(1+1/K_1)$ and by $\log_{10}\,(I_1/I_2-1)\,(1+K_1)$ respectively. In these expressions, $\Delta E + p\Delta V$ equals ΔH, the heat of reaction.

On the basis of experimental data, the sulfanilamide inhibition of bacterial luminescence conforms to Type I, with a heat and entropy of reaction practically identical in various species. The urethane inhibition conforms to Type II, with a heat and entropy of reaction depending on normal temperature relationships, varying widely in different species. Changes in apparent activation energies and optimum temperatures of luminescence, found in the experiments, are implicit in the theoretically derived expressions.

85. Characteristics of human sympathetic ganglion cells cultivated in vitro. M. R. Murray and A. P. Stout, College of Physicians and Surgeons, New York. (Lantern; 15 min.)

The observations to be described are based on normal and sympathetic ganglia from patients ranging in age from 16 to 66 years. This material was cultivated in vitro by the Maximow lying-drop method for periods longer than a month, during which time the explant remained in its original situation, and the morphology and physiology of the emigrating ganglion-cells was observed. These cells become dedifferentiated to a considerable extent, migrate away from the explant in a generally centrifugal direction, and dispose themselves in various conformations throughout the medium. They show some affinity for capillaries, which as empty endothelial tubes often grow out from the explant through the clot. Ganglion-cells from a 66-year-old man have been seen to divide mitotically under these culture conditions. After a period in vitro the cells tend to redifferentiate to some extent, sometimes regaining a little of their original pigment, which was resolved before their emigration. Neurofibrils are seen in fixed preparations. Growth cones, retraction clubs and other characteristic neural phenomena are habitually observed in the numerous dendritic extensions of these non-functioning cells.

86. Influence of the medium on radiation injury of sperm. T. C. Evans and J. C. Slaughter, University of Iowa, G. Failla, Memorial Hospital, New York, and E. P. Little, Marine Biological Laboratory. (Lantern: 10 min.)

The fertilizing power of a dilute suspension of sperm (Arbacia punctulata) is materially decreased by moderate doses of x-rays. The degree of this radiation effect is modified by: (1) the concentration of the sperm in the suspension, (2) the presence of certain proteins, and (3) the presence of other sperm (Nereis), during the irradiation.

These and other experimental findings may be accounted for on the assumption that the action of the radiation on the sperm is an indirect one, brought about through the intermediary of activated water molecules (originally postulated by Fricke).

Similar phenomena have been observed in the case of irradiated catalase solutions. The activated water molecule hypothesis is applicable to these results as well.

87. Changes produced in Arbacia sperm by immersion in irradiated sea-water. T. C. Evans, University of Iowa. (Lantern: 5 min.)

Immersion of Arbacia sperm (in low concentration) in heavily irradiated sea-water (100,000 r) and in very dilute solutions of hydrogen peroxide in sea-water

(1: 1,000,000) produce comparable effects. The effects studied are: (1) decrease in survival time of the sperm, and (2) cleavage delay in eggs fertilized therewith. It would seem, therefore, that these effects of irradiated sea-water on the sperm are due to hydrogen peroxide produced by the radiation. This view is supported by the titanic chloride test for hydrogen peroxide in irradiated sea-water.

88. Inhibitory effect of caffeine on oxygen consumption in Arbacia eggs. Ralph Holt Cheney, Long Island University. (Lantern: 15 min.)

This study of the micro-respiration of fertilized Arbacia eggs is one phase of a general investigation of the effect of caffeine upon the reproduction and growth of animals. Five-tenths of one cubic centimeter of a thirty-five per centum suspension of the fertilized eggs of Arbacia punctulata in sea-water was placed in each Warburg flask. Sea-water or the experimental concentration of caffeine-in-sea-water was added to each flask to maintain a total volume of 2 cc. of fluid in all vessels. Percentages of caffeine employed varied from 0.002% to 2.0%. Experiments were conducted at 25°C. Oxygen consumption was determined at regular intervals for a total of three hours by means of the Warburg manometers and method.

The results were expressed as a time relationship curve in cu. mm. of oxygen consumed over the three-hour period. The experimentals varied from non-caffeinized controls to the extent of 0.5% to 61.0%. Changes of less than 10% were considered not significant. Inhibition was demonstrated by concentrations equivalent to 0.1% caffeine or above. Cleavage stages were recorded in all control and experimental vessels at the termination of each experiment and checked against corresponding percentages in other parallel experiments of the series.

89. The effect of respiratory inhibitors on the oxygen consumption of Paramecium calkinsi. Edgar J. Boell, Osborn Zoological Laboratory, Yale University. (Lantern; 15 min.)

A study has been made of the influence of potassium cyanide and sodium azide on the oxygen consumption of Paramecium calkinsi. The results indicate that with dosages of cyanide, similar to those commonly used in respiratory studies, the oxygen consumption of Paramecium is depressed reversibly to a level approximately 50% of the normal. With sodium azide, a substance whose effect had not previously been studied on the respiration of ciliates, a similar reversible depression occurs.

In the presence of azide, an initial period of depressed respiration, lasting from 30 to 120 minutes, is followed by marked augmentation of oxygen consumption, the rate of respiration rising to a value two to three times that of the control. The increased respiration is different from that commonly obtained with low concentrations of poison in that it develops only after an initial depression. Although the nature of the extra oxygen uptake is obscure, studies of the respiratory quotients of normal and azide-inhibited Paramecia indicate that the combustion of organic metabolite is involved.

The reported failure of others to observe cyanide depression of respiration in Paramecium may possibly de due to the fact that in the use of cyanide certain technical details, whose importance has recently been emphasized, have been given inadequate consideration.

Insofar as susceptibility to cyanide and azide can be used as tests for the functioning in the cell of the cytochrome-cytochrome oxidase system, the results suggest that the respiratory mechanism of Paramecium resembles that of most animal and plant cells.

90. The action of various types of electric fields on the relative viscosity of the plasmagel of Amoeba proteus. Clifford A. Angerer and Karl M. Wilbur, Ohio State University and Dalhousie University. (Lantern; 15 min.)

This investigation is a continuation of an earlier report (Anat. Rec., 57: 52). The centrifuge method was employed in the determination of the viscosity of the cortical cytoplasm (plasmagel) of A. proteus. Amoebae were exposed to the following current densities for varying periods of time: 2.5 ×, 10 ×, 20 × and 30 × 10^{-3} amps./cm.^{2} for direct current and 2.3 ×, 5.4 × and 7 × 10^{-3} amps./cm.^{2} for alternating current. There is a prolonged time-interval (latent period) during which continuous exposure to relatively low "D.C." densities has no effect. Further exposure caused a decrease in viscosity to a minimum value characteristic of the specific current density employed. The two stronger current densities caused, ultimately, the characteristic anodal breakdown. Decreasing the electrolytic content of the immersion-fluid decreased the time of appearance of the various attributes of the response. The viscosity changes in the plasmagel on exposure to alternating current densities were essentially the same as for "D.C." with the following exceptions: (1) no latent period was detected when densities of relatively similar values were employed. (2) "A.C." fields are more effective in lowering the viscosity than are "D.C." fields. Tests with condenser-discharges of 2 and 4 mfd. capacity, respectively, produced a lowering of the viscosity of the plasmagel. Since the plasmagel has been shown to possess thixotropic properties it was of interest to determine the time-rate of recovery of the viscosity after removal of the electrical stress. Recovery of the "normal" viscosity value (160 units) after being experimentally lowered to the 80 and 60 units respectively for "D.C." and "A.C." occurred in ca. 4 minutes.

91. Fourteen-day rhythms in spiraling ratios of amebas and human leucocytes. A. A. Shaeffer, Temple University. (Lantern; motion picture projector; 15 min.)

When amebas and leucocytes are placed in capillary glass tubes of suitable size, they move freely in helical spiral paths. The direction of the spiral alternates frequently from right to left. To get the ratio of the amount of left path as compared with the amount of right path which is characteristic of an ameba or leucocyte, all the right and the left sections are mapped by means of a camera lucida for 150 minutes or longer if necessary to get an even number of complete right and left sections. The total amount of left path divided by the total amount of right path gives the left: right ratio.

Previous assumptions that the observed fluctuations in these ratios are due to cultural conditions, temporary physiological changes, etc., have been found incorrect. If spiraling ratios are obtained daily from an isolation pedigree culture, the ratios are found to be rhythmic with a period of 14 days. The amebas are first strongly left, but become gradually more right by .1 every day. Every

14 days a new rhythm starts. Other elements of pattern appear indicating other rhythms of different periods.

In human polymorphs similar 14-day rhythms appear but they are much less conspicuous, possibly because they originate in many different regions of the body and not from a single individual as in an isolation pedigree culture of amebas. Certain features of the leucocyte rhythms seem to be associated with general bodily reactions such as those incident to a common cold.

Parasitology, Morphology and Ecology (Sect. C)

92. A FOLLICULINID CARRIED BY LIMULUS. E. A. Andrews and T. C. Nelson, The John Hopkins University and Rutgers University. (Lantern; 15 min.)

Ciliated protozoans of the family Folliculinidae dwell much of the time in chitinoid tests attached to a great variety of objects. Species and genera are characterized largely by the features of their tests which persist long after the animals have left them. Some few species have been found only upon certain living crustacea; to such commensal associations we now add that of a folliculinid and an arachnoid, Limulus. During the past summer the junior contributor found that old female limuli on the coast of New Jersey when infested with the triclad Bdelloura candida and its relatives also carries folliculinids in quantity. The protozoa are attached not to the Limulus directly but almost solely to the margin of the egg capsules of the triclad. This association thus includes three members: the Limulus as host, the turbellarian as commensal, and the folliculinid as hypercommensal upon the capsules of the worm. These folliculinids are sufficiently different to warrant formation of a new genus to be described later. Sixty years ago Carl F. Gissler, Ph.D. described and figured them but deemed them part of the egg capsules. At the same time Dr. John A. Ryder also discovered them and thought them Epistylis or Zoothamnion (Vorticellidae). The former may also occur in numbers in addition to the folliculinids. So Wilhelmi published his colored sketches of capsule and folliculinids in his monograph of the triclads in 1907 without further identification. What becomes of them when the capsules disintegrate in winter remains to be determined.

93. THE OYSTER CRAB, PINNOTHERES OSTREUM, PARASITIC ON THE OYSTER. Leslie A. Stauber, New Jersey Agricultural Experiment Station. (Introduced by T. C. Nelson.) (Lantern, 15 min.)

Formerly widely considered a commensal, the evidence presented indicates that the oyster crab produces definite injury to the oyster and therefore is a true parasite. The invasive stages are post-planktonic males and females with a carapace from 1.5–2.5 mm. wide. Unlike the soft-bodied adult female, the only stage commonly reported from the oyster, the first stage crabs are well fortified with lime salts. The oyster strains silt and food organisms from the water and carries them toward the mouth partly in the marginal furrow of each demibranch. The oyster crab, situated usually in the mantle or infrabranchial cavity, feeds chiefly on these mucus-laden food strings as they pass anteriad. In so doing the chelipeds injure the delicate gill tissue and eventually cause significant destruction

to the gills. The erosions, involving at least 2 demibranchs, most frequently occur at the anterior end and may be caused by the activity of a single male or female crab although multiple infestations have been regularly seen. In one very weak oyster 262 first stage crabs were found. There is usually only one eroded area regardless of the number of crabs present suggesting that the others may enter after the initial lesion has weakened the oyster. All 5 growth stages of the female crab have been found, accompanied by gill damage. Oysters exposed to large numbers of the free-swimming first stage crabs have become heavily infested. Serious losses of oysters are reported.

94. The urogenital system of the male frilled shark, Chlamydoselachus anguineus Garman. Perry W. Gilbert, Cornell University. (Introduced by F. B. Hutt.) (Lantern; 15 min.)

Little is known about the male urogenital system of Chlamydoselachus, primarily because of the paucity of specimens of this rare, deep-water elasmobranch. The present study, based on four adult male specimens, figures and describes the gross structure of the testes and kidneys as well as their associated ducts. Both kidneys and testes are narrow, elongate organs conforming with the eel-like contours of the shark's body. The mesonephric ducts are but slightly convoluted at their anterior ends and receive segmentally, throughout the cranial three-fifths of their length, the collecting tubules from the kidney. The gross details of these tubules have been worked out using injections of latex, vinylite, and India ink. Caudally each mesonephric duct expands to form a seminal vesicle characterized by prominent internal fleshy transverse folds. An accessory mesonephric duct receives the collecting tubules from that part of the kidney dorsal to the seminal vesicle and opens into the mesonephric duct distal to a small sperm sac at the base of the urogenital papilla. Mesonephric ducts, accessory mesonephric ducts, seminal vesicles, sperm sacs and even the urogenital papillae are paired and appear to be developed about equally on either side. The prominent intromittent organs or claspers are unusual in that associated siphon sacs are absent. Instead, a "clasper cavity," analogous to a siphon sac, is located on the ventral side at the proximal end of either clasper groove.

95. The status of the modern Amphibia among the tetrapoda. F. Gaynor Evans, Duke University. (Introduced by A. M. Holmquist.) (Lantern; 10 min.) (To be presented by Wm. K. Gregory, Columbia University.)

In spite of the great amount of work done on the modern Amphibia their true status among the tetrapods has not been pointed out as clearly as it should have been. Paleontologists have long recognized that the living Amphibia are all highly specialized vertebrates but this fact does not seem to have been generally realized as a survey of recent Biology and Zoology textbooks will show.

The secondary specialized nature of the modern Amphibia is clearly indicated by comparative osteological studies of representative forms which were compared with the lizard Iguana on one hand and the labyrinthodont Eryops, a really primitive tetrapod, on the other. The amphibian types used were Rana, Cryptobranchus, Necturus, and Ichthyophis.

The results of such a comparative study of the skeleton show that in 30 features the modern Amphibia have departed more widely from the primitive tetrapod condition, as represented by the labyrinthodonts, than has a reptile such as Iguana. Many of these features in Amphibia are the result of the degeneration or loss of structures present in the primitive ancestral tetrapods and retained by Iguana.

96. Some unusual mammalian anomalies. Bert Cunningham, Duke University. (Lantern; 15 min.)

Two anomalies are presented. The first is that of two rats showing an unusual hermaphroditic condition in which ovaries were present, the unopened vagina was herniated forming a sac under the skin which gives the general appearance of a scrotum, and a well developed penis with an enclosed urethra also present. The ovaries were functional as evidenced by mature follicles and corpora lutea.

The second is a newborn sheep. The monster was without head and fore-legs. The posterior limbs appeared normal. A few vertebrae were present in the pelvic region, and there were possibly some poorly developed ribs. The abdominal viscera occupied most of the body cavity. Other anatomical features will be shown. The animal was apparently full term as evidenced by a full coat of wool, but was born dead.

97. Granular cells of the human juxta-glomerular apparatus. F. W. Dunihue, Medical College, University of Vermont. (Lantern; 15 min.)

In a few juxta-glomerular apparatus of "normal" rabbits there are cells which contain fuchsinophilic granules. These cells are increased in number following experimental procedures which induce renal hypertension. It has been suggested, although not proven, that these cells are secretory and play a role in the genesis of hypertension. The consensus appears to be that these cells are not present in man and experimental animals other than the rabbit; although there have been a few positive reports. This renders questionable the above suggestion, at least as a generalization.

This report affirms the presence of granular cells in the human juxta-glomerular apparatus similar to those found in rabbits. Correlation of the presence of these cells with any specific clinical entity is impossible at this time; therefore no new evidence is advanced in support of the suggested functional role of such cells. Their presence, however, removes at least one objection to this hypothesis.

A more detailed report will be made concerning the nature, occurrence, fixation and staining reaction of these granular cells.

98. Concerning the group survival value of the social peck order. W. C. Allee and A. M. Guhl, The University of Chicago. (Lantern; 15 min.)

Vertebrates which rank high in the social order of their group may thereby possess definite individual survival values as compared with low-ranking members of the same groups. An experiment was devised to ascertain whether organized flocks have group survival value when compared with another similar flock which is undergoing constant reorganization. In making the test, line-bred White Leghorn hens were arranged at random. Three flocks of seven each were allowed to establish and maintain a peck order. In a fourth flock, the longest resident was

replaced daily from a group of 21 isolated hens. After isolation for 21 days, these alternates meet former flockmates as strangers and the peck order is in a continual state of flux. This experiment has lasted for 19 weeks.

Except for the initial period when they were establishing their own social order, the controls pecked each other less and consumed more food than the experimentals. The controls maintained their body weight while the experimentals showed a fairly consistent loss. The experimentals regained body weight during isolation when their food consumption approached that of the controls. No marked difference occurred in egg production until the sixteenth week and the situation now is unsettled. The intensities of pecking and avoidance decreased among the controls and their pecks have lately tended toward being symbolic rather than being driven home as they still are in the experimental flock.

Under the conditions tested, the organized flocks show group survival values which, if they occurred in nature, might afford a basis for natural selection.

99. TETRALTERA, A NEW HOMOEOTIC MUTANT IN DROSOPHILA MELANOGASTER. Claude A. Villee, University of North Carolina. (Introduced by R. E. Coker.) (Lantern; 15 min.)

Homoeosis is the name given by Bateson to the alteration of one organ of a segmental series to resemble some other member of the series. In tetraltera, the wings are changed into halteres (balancers). The tetraltera gene was localized at 48.5 in the third chromosome. The phenotypic expression of tetraltera is variable; many intermediates between wing and haltere are found, or the wing may be reduced to a small stump or be completely missing. The members of the tetraltera series correspond closely with those of the tetraptera series described by Astauroff. The wings of the aberrant Dipteran Termitoxenia provide a taxonomic parallel to tetraltera. Morphological study of the tetraltera phenotypes revealed that the time of determination of the wing is later than that of the scutellum which in turn is later than that of the rest of the mesonotum. The formation of normal bristle pattern on the mesonotum depends upon the extension of each mesothoracic disc to the dorsal midline. The development of each side of the mesothorax is independent of the other. The penetrance of the tetraltera gene varies inversely with the temperature, from 35% at 15°C. to 1% at 29°C. Prolonging the time of early development by decreasing the food supply increases the penetrance. The mesothoracic discs are smaller and less differentiated than normal in both 96- and 72-hour larvae. The importance in evolutionary considerations of a gene-controlled embryological system capable of producing such large changes is evident.

100. THE BEARING OF THE GENOTYPE OF EXTREME VARIANTS ON SELECTION. H. D. Goodale, Mount Hope Farm. (15 min.)

A small proportion of the extreme phenotypic variants of a continuously varying character beget children more extreme than previous extremes. By selection of these genotypes, generation after generation, marked changes have been made in characters under selection. The rate of change is small. The exploration, even superficially, of evolutionary modification by this means is time-consuming, but offers hope of gaining further insight into the action of selection, artificial or natural. The small proportion of extreme variants which produce variants more extreme than previous extremes may explain the lack of progress in many selection efforts.

PAPERS TO BE PRESENTED BY TITLE

Cellular Physiology

122. Histological studies of vitamin E deficient testes of the fowl. F. B. Adamstone, University of Illinois.

Earlier studies showed that male fowl reared on a vitamin E deficient diet from 6 weeks after hatching became sterile after prolonged feeding. The testes, however, appeared to be very resistant to E-deficiency and sweeping degenerative changes similar to those of the rat were not encountered.

In the present studies the chicks were reared from hatching on the deficient ration. In those which survived long enough more conspicuous injury was observed. The first recognizable injury was damage to the nuclei of the primary spermatocytes. In these the chromatin was shrunken to one side and densely clumped. This injury appears to prevent the further maturation of germ cells since where it occurred individual Sertoli cells with their complements of maturing germ cells stood out clearly isolated from one another.

In more advanced E-deficiency a condition somewhat similar to Mason's stage I (in the rat), with fused sperm, was encountered followed by the beading of tubules characteristic of his stage II. Giant cell formation (stage III) was a very prominent feature of the degenerating tubules. Giant cells were formed by spermatids, by II-spermatocytes and by I-spermatocytes. Large numbers of nuclei, often as many as 500 or more were present in the giant cells. The injured I-spermatocytes formed a distinctive, easily-recognized type of giant cell. Denuding of the germinal epithelium finally occurred and the degenerate testis was converted into a watery sac which collapsed on puncture. The whole process is thus seen to be very similar to that which occurs in the rat.

123. The effects of vitamin E deficiency in the guppy, Lebistes reticulatus. H. W. Cumings, University of Illinois. (Introduced by F. B. Adamstone.)

Studies on vitamin E deficiency extended to the guppy show that this fish requires vitamin E for normal maintenance just as certain birds and mammals.

Two groups of fish reared on synthetic and iron-treated rations from birth and from 45 days after birth were used in the experiments. A whole series of reactions was obtained in both cases regardless of type of diet. Growth was seriously inhibited and in some cases even stopped. Muscular dystrophy occurred with unfailing regularity and in some cases the muscles were almost entirely destroyed. The reproductive system was studied in the males and it was found that the testes either failed to develop at all, or, if spermatogenesis had begun, degeneration set in affecting the more mature germinal elements first and later the younger stages. In the visceral organs injury to the liver, pancreas and spleen was frequently encountered often marked by clumping of the chromatin of the nuclei or the formation of signet ring nuclei. A characteristic reticular tissue replaced the degenerate tissues. Large extraneous masses of the same tissue developed in the gut, in some specimens, sometimes blocking the lumen of the tube.

Observations on the two groups of fish indicate that the severity of injury is correlated with the age at which use of the E-deficient diet was begun for fish placed on the diet at 45 days showed much less injury.

That vitamin E-deficiency was responsible for the reactions encountered was proven by failure of the symptoms to develop when α-tocopherol was used as a dietary supplement.

124. Development of Arbacia eggs in caffeinized sea water. Ralph Holt Cheney, Long Island University.

One phase of a study to determine the effect of caffeine upon reproduction in animals was investigated by subjecting fertilized eggs to caffeinized sea water during their cleavage stages. Nine concentrations of caffeine-in-sea-water were employed corresponding to percentages varying from 0.002% to 2.0%. Experiments were conducted at 25°C. Observations of the eggs were made at intervals of 10, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180 minutes; and then, at longer periods until either pluteus larvae developed or cytolysis occurred.

Plutei developed in normal time and form in all combinations of caffeinized eggs and sperm in a medium containing 0.004% or less caffeine-in-sea-water. If the environment was the equivalent of 0.02% to 0.04%, plutei were normal but developed in delayed time. In 0.1% to 0.2%, cleavage was arrested at various stages. Higher percentages caused progressively abnormal cleavages and the arrest of division at increasingly earlier stages.

125. Determination of polarity in Fucus eggs by gradients of dinitrophenol: a new method for establishing steep gradients across living cells. D. M. Whitaker and W. E. Berg, Stanford University.

A drop of sea water containing Fucus eggs (diameter 75 μ) was quickly mixed with a drop of 2–3% sea water agar in a small warmed vessel. The agar was slightly above the congealing temperature. A collar-like section of 2 cm. glass tubing was cut about $\frac{1}{8}$ inch long. One end of this collar was dipped in the mixture so that a thin film or membrane of agar formed across it, resembling a soap bubble film. The film was formed 1–2 seconds after mixing the eggs, which were not damaged by the brief high temperature.

The film across the collar contained evenly dispersed eggs firmly embedded. The central part of the film was uniform in thickness, which could be varied (e.g. 30–150 μ) by timing and temperature. Multiple layers build up if dipping is repeated. Gelatin or gelatin-agar mixtures can be used.

The collar containing the film was inserted in a hole (ground fit) in a glass (or lucite) partition between two reservoirs containing flowing solutions. Extremely steep concentration gradients were established across eggs in the film.

With flowing sea water on one side of a 100 μ film, and $3–5 \times 10^{-5}$ M. dinitrophenol on the other, more than 90% (99% in some experiments) of the eggs formed rhizoids on the side toward the dinitrophenol.

The method is applicable to many types of cells and to any chemical solutions, as well as to temperature gradients and gas mixtures.

Cytology

126. Amitosis in the malpighian tubules of Melanoplus differentialis (Acrididae). Leonard G. Worley, Brooklyn College and the University of Nebraska.

A study has been made of the growth of the malpighian tubules in Melanoplus differentialis Thomas. Observations of the tubules made during the penultimate stage (instar vi) reveal many binucleate cells. Further examination indicates that this binucleate condition is attained through the failure of cytokinesis to keep pace with the nuclear divisions that accompany the differentiation of the tubules. Moreover, it is noted that karyokinesis is strictly amitotic. The nuclei first elongate and then, through medial constriction become dumb-bell shaped. Finally, the two halves pinch apart. All of this is accomplished without rupture or dissolution of the nuclear membrane and without the participation of the chromatin in any active manner; the nuclear contents remaining entirely passive during division. Mitotic division has never been observed in the malpighian tubules. The amitotic divisions take place largely in the longitudinal plane and provide the typical means of tubule elongation associated with the growth of the insect. Both nuclear and cytoplasmic divisions apparently cease with the attainment of the adult condition with the result that many of the adult cells are left permanently binucleate.

127. The mitotic index in the neural tube of the 48-hour chick as determined by the use of colchicine. T. M. Woodard and Sarah B. Estes, Vanderbilt University.

The percentage of each phase of mitosis of the total number of nuclei counted was determined in the neural tube of chicks treated with 1 mg. of colchicine for 20 minutes to 3 hours and compared with that of controls. The percentage of metaphases in treated chicks rises to a maximum of 21.2 in 3 hours. The percentage of each of the other phases declines to zero, and the total of all phases corresponds to the M. I. at any given time. This is 13.6 at 40 minutes, 15.9 at 1 hour, and 16.9 at 2 hours. Controls show an average M. I. of 9.3. These results show that: (1) the effect of colchicine is to accumulate at the metaphase stage all mitoses in progress; (2) between 9.3 and 21.2% of the nuclei are in some phase of mitosis within the experimental period; and (3) the true M. I. (percentage of nuclei in any phase of mitosis, including the visibly unrecognizable prophase) is much higher than has been recognized heretofore. It is adequate to account for the known increase in number of nuclei between any two stages in which counts of total number of neural tube nuclei have been made. It is, therefore, unnecessary to assume any other form of nuclei division in the neural tube other than the mitotic one.

128. The time and rate of mitosis in corneal wound healing. L. B. Arey, Northwestern University Medical School.

The details of epithelial repair and regeneration have been studied in a graded series of small wounds inflicted under controlled conditions on the cornea of the white rat. As is common in the healing of small wounds, mitosis enters as a late

factor after primary closure has been accomplished in a few hours by the movement of epithelial cells over the defect. The number of mitoses per cubic millimeter of corneal epithelium has been counted (and calculated) in stages ranging from 6 to 240 hours after wounding. The number of mitoses per cubic millimeter of normal, uninjured corneal epithelium is about 1650; this is nearly four times as great as occurs, for example, in the deeper layers of the oesophagus.

In the wound area the number of mitoses was reduced to one-half normal for some 72 hours. At 96 hours the normal rate was regained, and at 120 hours the rate rose 50% above normal. This was followed by a decline to normal at 192 hours, while at 240 hours the rate was somewhat subnormal. These time and rate relations represent new, quantitative information on the role of mitosis in restoring a tissue depleted by cell emigration in order to repair traumatic loss. Their interpretation and significance can easily be deduced by the reader.

129. On the nature and significance of grooved nuclei, especially in Brenner tumors. L. B. Arey, Northwestern University Medical School.

Grooved or folded nuclei are occasionally found in representatives of all the tissue groups. In some nuclei, as those of the Sertoli cells, longitudinal grooving is frequent and characteristic. A striking illustration is furnished by the relatively recently described Brenner tumor of the ovary. Here as high as 90% of the elongated, ovoid nuclei may show a longitudinal furrowing that gives an appearance much like a grain of puffed wheat. Possible explanations which can be rejected, of the cause of this specialization include: pressure deformation; amitotic activity; and nuclear regression. More appealing is the interpretation that such alterations in form are functional adaptations. Often there seems to be a relation to active or forced metabolism. The fact that the writer has traced the origin of a Brenner tumor directly to the germinal epithelium of the ovary directs future attention to the Brenner-tumor cell in relation to estrogenic output. Although proof of the physiological activity of this tumor has usually been said to be lacking, there are some data which correlate its presence with such phenomena as post-menopausal uterine bleeding and other changes usually associated with the presence of estrogens.

130. Endomitosis (?) in grashopper serosa cells. T. N. Tahmisian, T. H. Allen and J. H. Bodine, State University of Iowa.

The serosae of Melanoplus differentialis eggs were dissected free under 0.9% sodium chloride then (1) stained with aceto-carmine, (2) fixed in Champy's and incubated in 2% osmic acid, (3) fixed in Bouin's and stained with Heidenhain's hematoxylin, or (4) fixed in Gilson's and stained with Feulgen's fuchsin-sulfurous. Serial sections and whole mounts were prepared.

Intranuclear chromatic phenomena seem to occur in prediapause yet not in diapause serosa cells. The nuclei contain dispersed chromatin granules. Some have one small thread-like aggregation of chromatin. Others have a shorter, thicker chromatic body. In a third type this chromatic body appears to have divided longitudinally in such a way that the two parts are joined by thin fibers. These bodies in a fourth type are distinctly separate. All these structures

are Feulgen's positive, and at least one of these four types was seen in every nucleus aged 5 to 19 days. Therefore, it seems that a localized doubling of the chromatin content occurs in these nuclei. No spindle is detectable, and the nuclear membrane remains intact. Perhaps such changes may be called "endomitosis." If so, distinctions ought then to be made between general and local endomitosis.

131. Cytological studies of hyperplastic livers in the rat. Norman M. Sulkin, State University of Iowa. (Introduced by H. W. Beams.)

This investigation was undertaken to compare the nuclear cytology in the hepatic cells of the normal and restored liver of the rat. When two lobes of the liver are extirpated, the remaining lobe increases in size and a change in the nuclear picture occurs, whereas if one lobe is extirpated no such increase and change are observed.

Measurements of nuclei in normal liver produce a 3-mode curve in which approximately 85% of the nuclei are tetraploid, 10% are diploid, and 5% are octoploid, whereas measurements in the restored liver produce a 5-mode curve with an increase in the magnitude of polyploidy. The percentage of binucleate cells dropped from 11.7 $\pm$ .36% in the normal liver to 4.95 $\pm$ .24% in the restored.

Observations were also made in the area surrounding the central vein. Here, both in the control and restored livers, there was a significantly greater percentage of binucleate cells and an increase in degree of polyploidy. This suggests that division occurs more frequently in the area of the central vein.

Mitotic divisions have been observed both in the restoring liver and in the control.

These observations also support the view that inert materials may cause the failure of the cytoplasm to divide during karyokineses, since storage of glycogen first takes place around the central vein and is given up in the reverse order.

132. Segregation and behavior of spermatozoa in Terebra dislocata. T. M. Woodard, U. S. Fisheries Bureau, Beaufort, and Vanderbilt University.

The seminal receptacle of Terebra contains only typical spermatozoa, but both types of sperm are found in the male sperm duct and in the uterus. The typical sperm taken from any of these sources aggregate head-to-head in sea water. The aggregates formed of typical sperm alone, however, are less coherent than those formed in the presence of the atypical type and break down into smaller, more numerous aggregates. This difference is due to the presence in the one case of a felt of atypical sperm which in some way (perhaps mechanically) serves to maintain the aggregates.

Ecology

133. Geographical distribution of the Folliculinidae. E. A. Andrews, The Johns Hopkins University.

Ciliated protozoans of the family Folliculinidae may well be world wide in distribution, but as yet they have not been recorded from Africa, the greater part of Asia, and the Americas, save only Uraguay and the coasts of Massachusetts, Rhode Island, Maryland and North Carolins.

The tests they live in are fastened to diverse objects and study of shells, chiefly oyster shells, collected the past summer through the courtesy of several zoologists has extended the known distribution of the Folliculinidae to the following localities: Boothbay Harbor, Maine; Waquoit Bay, East of Woods Hole, Massachusetts; Long Island Sound, shores of New York; Delaware Bay, shores of New Jersey; Lower Chesapeake Bay, shores of Virginia; Atlantic waters opening from the eastern shore of Virginia: and in the Gulf of Mexico; Florida shores at Cedar Keys and near Pensacola; Grand Pass, Louisiana; Near Rockport, Texas: in the Pacific on the shores of California: in the Gulf of St. Lawrence, shores of Nova Scotia and of Prince Edward Island, Canada.

The percentages of random shells inhabited by Folliculinidae was seldom large and often very small.

On any one shell they rarely stand isolated but generally in settlements of from few to 500.

Thus far but five or six species have been found and these were known in Europe.

134. Plankton organisms where fresh and salt waters meet. R. P. Cowles and C. E. Brambel, The Johns Hopkins University.

It is generally assumed by oceanographers that there is a high mortality of plankton organisms where fresh and salt waters meet. For this reason, we have investigated the distribution of the plankton alga, Scenedesmus quadricauda in Back River, Maryland.

We have found that in the upper part of that river where Scenedesmus lives by the million in one liter of water the highest counts are in the fresher water. Samples taken at intervals down the river toward the more saline Chesapeake Bay show a rather sudden decrease in numbers of these organisms till in the bay none are to be found. These observations, contrary to the experiments of Finley, suggest that higher salinities kill off Scenedesmus.

The almost total absence of Scenedesmus in the lower part of Back River and Chesapeake bay, and especially its sudden disappearance after counts of a million or so in the upper part of Back River suggest that there may be some toxic substances, salt or some other compounds, coming in from the bay.

However, chemical tests show also that in the upper part of Back River the phosphates and nitrates are especially high. This may account for the large numbers of Scenedesmus. Furthermore, the values for these same nutrient salts decrease passing toward the mouth of Back River, with the decrease in counts.

These three factors, with possibly others, need further investigation from chemical and physiological points of view.

135. Studies on the black sediment in Back River, Baltimore, Md. C. E. Brambel and R. P. Cowles, The Johns Hopkins University.

Bottom samples taken from Back River are composed of a black, unctuous, water logged mud with a disagreeable odor. On analysis, these samples yield about 98% water and 20% organic matter in the dried residue. Ferrous iron (10% to 15%) is present in contrast to ferric iron found in the suspended matter. A strong reducing reaction has been obtained with indicators and electrometric methods.

Laboratory studies reveal that samples of water kept in sealed containers and in the dark develop a black gelatinous sediment similar in physical properties to that found in the field. Sealed samples kept in the light develop a reddish-brown sediment with numerous green micro organisms (notably Scenedesmus and Pediastrum). Tests with redox indicators show the black sediment produced in the laboratory to be in a reducing condition. The iron changes from ferric to the ferrous state. Furthermore, these experiments indicate the presence of micro-organisms capable of producing H_2S.

On the basis of the foregoing observations, a cyclic change from the oxidized to the reduced state occurs reversibly with respect to iron in Back River. The suspended particles containing adsorbed ferric iron drop to the bottom into an environment of negligible oxygen and rich H_2S content.

136. Growth of the oyster drill, Urosalpinx cinerea, Say, feeding on four different food animals. James B. Engle, U. S. Fish and Wildlife Service, Milford Biological Laboratory. (Introduced by V. L. Loosanoff.)

Observations on four groups of drills each group feeding exclusively on a selected food organism demonstrated that considerable variation in rate of growth between groups occurred.

The foods, Ostrea virginica, Mya arenaria, Mytilus edulis and Balanus sp., were secured from the same locality as the drill and, therefore, with the possible exception of Mya, were its natural prey. Excess food was available at all times. Observations were carried on for 13 months although the drills fed only the 7 months between May and November.

Soon after the snails began to feed, a difference in growth rate became apparent. With Mya as food Urosalpinx made the greatest increase. During the same period snails feeding on Ostrea were next. Snails eating Balanus grew slightly less than those on Ostrea. Drills eating Mytilus showed the least growth.

Drills fed Mya produced their best growth between July 12th and August 10th; those eating Ostrea from August 10th to September 6th when the group eating Balanus also made its maximum increase. Drills eating Mytilus grew best from June 12th to July 12th.

Partial substantiation of the above results were observed in the field where all these animals were intimately associated. The area where the experimental drills were collected was in the midst of an extensive Mytilus bed. Examination of that area showed very few Mytilus killed by drills, while young Ostrea attached to the rocks, or shells of living and dead Mytilus, were readily eaten. Large numbers of Balanus were also destroyed.

137. Tolerance of the Gulf Coast snail, Thais floridana, to waters of low salinity. Victor Schechter, The City College of New York.

Thais floridana is regarded as a serious natural enemy of the oyster all along the Gulf Coast. Inasmuch as both the snail and the oyster inhabit shallow coastal waters which are subject to wide variations in salinity, it is of practical as well as theoretical significance to know the relative extent of dilution which these animals can withstand. It was found in the experiments that lethal effects set in rapidly when dilution drops below approximately 10 parts of salt per thousand.

Below 7 p.p.M., due apparently to the secondary effect of causing closure of the operculum, the lethal effect is somewhat lessened. In all dilutions small snails were more susceptible than large.

138. Comparative effects of low humidities on water loss and reproductive capacity of two species of saturniid moths. Daniel Ludwig, New York University, University Heights.

Pupae of the Luna moth, Tropaea luna, and the Cynthia moth, Samia walkeri, were brought from out-of-door conditions during the winter and kept at different relative humidities at 25°C. until adult emergence. In both species loss of weight occurred at all humidities and was greater the lower the humidity. Luna pupae lost weight at a regular and rapid rate until emergence, which in this species occurred in approximately 3 weeks. Cynthia pupae lost weight very gradually for a period of approximately 6 weeks during which they remained in winter dormancy. This period was followed by another during which loss of weight was more rapid and which continued until adult emergence approximately 80 days after the beginning of the experiment. Cynthia pupae in comparable physiological stages and under the same humidity conditions are more resistant to desiccation than Luna pupae. Furthermore, pupae of the former species can be desiccated to a greater degree before there is a decrease in the egg production of moths which develop from the dehydrated pupae. A pupal weight loss of more than 15% resulted in a marked decrease in oviposition by Luna moths, while no corresponding decrease was noted in Cynthia moths until pupal weight loss amounted to approximately 30%.

139. Life history of Hurter's spadefoot. Arthur N. Bragg, University of Oklahoma.

Scaphiopus hurterii was watched in breeding congresses and the eggs and tadpoles were collected and studied. The breeding behavior resembles that of the eastern spadefoot (S. h. holbrookii (Harlan)) more than the prairie and desert forms (S. bombifrons Cope, S. hammondii Baird, and S. couchii Baird). The eggs are black and white and in the jelly superficially resembles those of Bufo. The embryo is black but the early tadpoles become a dark grey. Development is rapid. The tadpole hatches in a premotile condition and by a process essentially like that in Bufo w. woodhousii Girard. Tadpole development is rapid and metamorphosis occurs in about 1 month. The tadpole is very small at this time and has the characteristic crescent-shaped light patches of the adult, dorsally, before leaving the water. The labial formula is 3/4 with some variations in the exact arrangement of the dorsal rows.

Metamorphosis is accompanied by a social aggregation of tadpoles of a type apparently never before described. From these aggregations they leave the water singly and will die on the bank from desiccation if not protected by leaves or other cover. They do not burrow at this time.

They feed immediately on small insects and may grow to one-third or one-half size during the first summer. They tend to be diurnal during the first few weeks out of water, but are very secretive and nocturnal after this.

140. The habits of spadefoot toads in relation to their phylogeny. Arthur N. Bragg, University of Oklahoma.

Eight spadefoots occur in North America, four in the subgenus Spea, four in Scaphiopus (Tanner). Four occupy Oklahoma (two Spea and two Scaphiopus). A field study of the latter forms, correlated with data on others from the literature, allows an interpretation of phylogeny based upon habits as well as morphology.

Spadefoots arose in the southwest and developed habits adaptive to desert regions. The line then bifurcated, members of Spea and one species of Scaphiopus (couchii) remaining in xeric environments and retaining xeric habits: others spread eastward modifying the primitive habit-pattern slightly in more mesic situations. At present, S. couchii is morphologically a member of the eastern group: in habits, clearly western.

This shows that, in evolution, changes in habits do not necessarily follow morphological changes and that it is unsafe to generalize about habits on the basis of morphology alone. Habits are more easily changed by new and different environments than is morphology. An incidental result of this study explains the mystifying behavior of the solitary spadefoot of the east; for, here is an amphibian adapted for desert-life now occupying the moist woodland in company with primitively mesically adapted species. This interpretation implies that a xerically adapted amphibian changes less in habits in spreading to more mesic environments than under reverse conditions. Environmental factors are still of great importance to phylogenetic studies.

141. The pyramid of life in some inland lakes of Wisconsin. Chancey Juday, University of Wisconsin.

On a live-weight, ash-free basis, the ratio of the weight of the plants (phytoplankton and large aquatics) to that of the animals (zooplankton, bottom fauna and fish) fell between 6 and 6.8 in two soft water lakes. Thus the plants, which represent the actual and potential food for all animals, contributed 83% to the pyramid of life in one of these lakes and almost 86% in the other. Exclusive of fish, the ratio of the weight of the plants to that of the animals averaged 7.3 in one and 7.5 in the other as compared with 12.1 and 22.2 in two hard water lakes. The soft water lakes, therefore, were approximately two to three times as efficient in converting the plants into animal material as the hard water lakes, but the standing crops of plants in the latter were three to five times as large per unit of area as in the former. The hard waters on the other hand yielded only one and a half to two and a half times as much animal material per unit area as the soft waters. Fish contributed only a small cap to the pyramid of life in the two soft water lakes; they comprised 1.2% of the weight of the biota of one lake out of a total of 1872 kg./ha. and 2.5% in the other in a total of 1410 kg./ha.

142. Social behavior of the western banded gecko. B. Greenberg, American Museum of Natural History and The State University of Iowa. (Introduced by P. L. Risley.)

More than 100 geckos (Coleonyx variegatus Baird) were studied in order to determine the social behavior patterns and the functions of certain secondary sex

characters (femoral glands, spurs, subcaudal sacs) common to many species of geckos. The mating pattern of the male was found to be a timed sequence of events in which each of the above epidermal specializations has a definite place. The male's copulatory reflexes elicit corresponding reflex reactions of the female, whose cooperation is essential for copulation. "Sex recognition" in this nocturnal species depends primarily upon behavioral cues. Anaesthetized males are treated as females but experiments concerned with discrimination between a male or female member of an introduced pair suggest that other stimuli may be present which involve the chemical senses. Absence of sex dimorphism of size and display characters has apparently not favored the development in the female of fight, dominance or territory. Thus Coleonyx differs from diurnal lizards such as Anolis where dimorphism is present and is accompanied by fighting among the females resident within a male's territory. Two opposing forces are seen to regulate the social system of the banded gecko. Dispersal during the breeding season is furthered by fighting among the males which leads to dominance and perhaps territory and by courtship of anestrous females. Aggregation as manifested under laboratory conditions is a strong factor even during the breeding season, when it probably aids reproduction, since the male's initial courtship performance is at close contact rather than at a distance.

143. Dominance, social order and territory in the lizard (Anolis carolinensis). B. Greenberg and G. K. Noble, American Museum of Natural History and The University of Chicago. (Introduced by W. C. Allee.)

Among groups of males kept in small cages (24 inches × 18 inches × 18 inches), a single male Anolis invariably dominates all the others. With greater available space, two males often divide a large cage (41 inches × 33 inches × 70 inches) into discrete territories; each is dominant in his own half. The dominant males seem to react definitely at territory boundaries. Given still more freedom (a greenhouse room), the males distribute themselves over extensive areas which they defend. Fights between adjacent individuals lead to the establishment of a dominance order which resembles the "peck right" order among hens. The defeated male continues to defend his territory except that he runs at the approach of his more aggressive neighbor. In one such case under observation, M2 on area II dominated both M1 (even on area I) and M3 (on area III) while at the same time M1 dominated M3 on area III and each of the latter two, at the fringes of their own territories, suppressed several other males. This is concluded to be "peck right" on a spaced or territorial basis. Females also fight among themselves in a male's territory and the resultant order gives a straight-line hierarchy.

A greater correlation between weight and dominance was found during than out of the breeding season. Since size and weight are directly correlated with age, sexual maturity and experience, it is suggested that the older, larger and more experienced males tend to be dominant during the breeding season.

144. Some aspects of the dominance-subordination relationship in Platypoecilus maculatus. James C. Braddock, The University of Chicago and Kansas State College. (Introduced by W. C. Allee.)

The poecilid fish, Platypoecilus maculatus, exhibits in its social behavior a dominance-subordination relationship qualitatively similar to that reported for the

common domestic fowl. The relationship is less stable in the case of Platypoecilus and is limited to a smaller number of individuals.

These experiments dealt with groups of four fish either in homosexual groups of four males or four females or in heterosexual groups of two males and two females. An effort was made to maintain similar general environmental conditions.

The experiments indicated the existence of social relationships in such small groups. These were of the hierarchal nip-right, type and were the expression of individual, contact-pair reactions. Quantitative differences exist between the social orders of males and females in homosexual groups; the latter are the more stable of the two. The heterosexual hierarchies are least stable. Straight-line orders occurred in all three sexual groupings, but were not the only type observed. There is evidence that contact-pair relationships rest upon individual recognition rather than innate aggressiveness.

Dominance appears to be based largely upon such physical factors as size, maleness, and aggressiveness as expressed by nipping. Certain psychological factors, such as acquaintance with an environment and position in an established society also enter.

No evidence of territorial behavior was noted.

Embryology

145. Regeneration and transplantation in Clymenella torquata. Effects of pieces of posterior nerve cord inserted in the open, terminal wounds of worms cut to regenerate anteriorly. Leonard P. Sayles, The City College of New York.

In 158 experiments with Clymenella, anterior regeneration was initiated by the removal of five head segments. Pieces of posterior nerve cord were then so implanted that their posterior ends were exposed at this terminal, regenerating region. Fifty-seven worms survived 10 days or more. Nine of these developed excellent head buds and seventeen others formed fairly well differentiated heads. Most of the buds of the latter group were bent considerably toward the side where the implant was located, apparently as a result of mechanical interference with the proper distribution of new material to that side. Fifteen produced only irregular masses of new material. Two formed head buds with small, extra cones of new near the tips of the implants. Seven produced head buds bearing partial tail buds near the implants. In five cases the buds were probably weakly developed heads but each included an extra, partial tail bud arising near the point where the posterior tip of the implant showed. The remaining two buds terminated in partial anal segments with no head features evident.

It has previously been shown that pieces of posterior nerve cord implanted in the anterior, head-forming region of intact hosts induce tail buds, but never heads. The present experiments demonstrate that implants of posterior nerve cord are also able to exert their strong organizing influence even upon the new material which is being produced in a region where the head-determining factor has been strengthened as a result of anterior regeneration.

146. Phenogenetic studies in the bar series of Drosophila melanogaster. Howard L. Gravett, Elon College. (Introduced by W. M. Luce.)

An attempt was made to correlate the embryological development of the optic buds in the bar series of Drosophila melanogaster with previously collected data on temperature-effective periods. Eggs of closely inbred stocks of wild, bar, infrabar, and double infrabar, collected under carefully controlled egg-laying conditions, were allowed to develop at 17, 22, and 27°C. Larvae were collected at 12-hour intervals at 17°C. beginning with the 132nd egg-larval hour and continuing through the 252nd hour. Collection at 22°C. started at the 72nd egg-larval hour and continued at 6-hour intervals through the 120th hour. Those larvae at 27°C. were also collected at 6-hour intervals beginning with the 48th hour and continuing through the 90th egg-larval hour. Averages of areas and averages of volumes of the optic buds were calculated for each egg-larval age group. The beginning of the temperature-effective period, the first appearance of the antennal bud, and the period of most rapid growth were correlated quite definitely in most of the stocks at 17, 22, and 27°C. No definite correlations with the end of temperature-effective period were apparent. It is hoped that the correlative information concerning the beginning of the temperature-effective period will be of some aid for a better understanding of development and genic action in general.

147. Effect of double matings on the fertility of a mutant of Drosophila melanogaster. R. C. Anderson and C. P. Oliver, University of Minnesota, Minneapolis.

Homozygous glossy females lack spermathecae and parovaria but the ventral receptacle seems to be normal. The infertile females produce an average of only ten offspring per breeding female; and most of the offspring are produced during the first day after the mating; a few occur during the second day, rarely during the third. Sperm can be observed in the ventral receptacle for at least 11 days; but in contrast to the normal females, the sperm gradually lose their motility; after the sixth day, no motile sperm are observed. Glossy and normal females were mated to normal males for 48 hours and then isolated for 5 days. On the eighth day, they were mated to males carrying dominant markers, hairless and moire. Control females produced offspring which were the results of sperm from both matings, with approximately an equal number from each mating. Offspring from glossy females were produced only from sperm of the second mating, the number of offspring being less than in the controls; offspring occurred only during the first 2 days following the second mating. The infertility of glossy females seems to be associated with inviability of sperm in the females rather than with egg disturbance. A second supply of sperm initiated a second productive period. Glossy females were also kept with males for various periods of time; and the longer periods of association gave a like increase in the number of offspring; a longer association apparently allowed more frequent insemination.

148. Induced haploidy and triploidy in Californian triturus. Donald P. Costello, University of North Carolina.

Using the refrigeration technique of Fankhauser and Griffiths ('39), eggs of Triturus similans (granulosus) were treated at varying periods after insemination.

Of 100 treated eggs surviving to the stage of tail-clipping, 13 were triploid, 81 diploid, 4 haploid, and 2 haplo-diploid. Fankhauser and Griffiths suggested the retention of the second polar body as the cause of triploidy, but offered no explanation of haploid-production.

One observation possibly elucidating the origin of such haploids is here recorded. Triturus rivularis eggs were inseminated with Triturus torosus sperm and refrigerated (lightly frozen) for the purpose of producing super-hybrids. No larvae with accentuated maternal characteristics were obtained, but two larva (of twenty) differentiated a pigment pattern remarkably like that of T. torosus. These larvae were oedoematous, and of characteristic haploid appearance except for large pigment cells. They did not feed at the proper time, and, as one died, the other was fixed and sectioned. Nuclear size was approximately the same as for normal hybrids; no mitotic figures suitable for chromosome counts were found. The androgenetic pigment pattern might be accounted for by the hypothesis that cold completely destroyed the second maturation spindle, the egg developing under the influence of the sperm nucleus. If these embryos were diploid, as the nuclear size suggests, there must have been a doubling of chromosome number by some unknown mechanism. Haplo-diploidy in Triturus similans supports this hypothesis.

149. Neural crest transplantations between Hyla and Triturus embryos. Donald P. Costello, University of North Carolina.

Baltzer ('41) on the basis of neural crest transplants between embryos of Hyla and urodeles (Amblystoma, Triton alpestris and palmatus) concluded that the donor pigment cells migrate out into the host tissue and there assume a pattern characteristic of the host. This contrasts with all earlier results of transplants between urodeles, in which the pattern formed by the migrating melanophores is distinctively donor.

Triturus torosus is better suited to these experiments than the European forms, since the larva possesses a distinct dorsal pigment band. In transplants of Hyla neural crest to T. torosus, typical Hyla melanophores (and guanophores) spread down the sides from the transplanted region, forming a scattered pattern similar to that of Hyla. No suggestion of band-formation by the Hyla melanophores was observed. The yolk zone of the host, however, constitutes a barrier to further melanophore migration.

In reciprocal transplants, the donor T. torosus pigment cells in the Hyla host never assume a typical T. torosus pattern. These dermal urodele melanophores are scattered in the Hyla epidermis, as Baltzer records, and do not form a dorsal band. Probably the difference in the skin structure of larval anura and urodeles is responsible for this difference in the results of reciprocal transplants.

150. Lacrimal duct determination and development in transplanted R. catesbeiana integument. O. M. Helff, New York University.

Autoplastic, reciprocal transplantations between integument of the back and that of the potential lacrimal duct region were made on premetamorphic larvae of R. catesbeiana. During larval metamorphosis, the transplants were removed and sectioned for detection of possible lacrimal duct development.

Sections of back-skin transplants, in potential lacrimal duct regions, exhibited no histological signs of lacrimal duct formation. Conversely, sections of potential lacrimal duct region integumentary transplants, on the back, gave evidence of pronounced lacrimal duct development. Data were also obtained which confirmed previous unpublished findings relative to the normal histogenesis of the lacrimal duct in this species.

The results indicate the following conclusions:

1. The origin of the lacrimal duct is chiefly from cells of the lower epidermal layers, which proliferate, basally, through a break in the basement membrane. The point of first proliferation is approximately halfway between the naris and the eye. There is apparently no cellular proliferation from the naso-lacrimal groove, as in the case of E. bislineata.

2. A narrow strip of epidermis running from the naris to the eye is determined, as regards subsequent lacrimal duct development during metamorphosis, for some time prior to the onset of larval involution. Actual histogenesis occurs, first, at a mid-point and then progresses anteriorly and posteriorly to complete the length of the duct.

3. There are no neighborhood inductive or predisposing factors essential for the normal development of lacrimal duct structures in R. catesbeiana.

151. Colchicine and limb regeneration in larval Amblystoma. C. S. Thornton, Kenyon College.

Amputated fore limbs of Amblystoma larvae, maintained in colchicine solutions of 1: 1000 or 1: 1500 dilution, fail completely to regenerate. Furthermore, colchicine-treated limbs undergo a progressive dedifferentiation which involves all the structures within the limb stumps. Due to the continued dedifferentiation of its tissues the limb stump becomes progressively reduced in size, until by 20 days after amputation and the beginning of the colchicine treatment the limb is represented only by a small mound on the side of the animal. Examination of sections through the limbs undergoing regression shows that the cartilage dedifferentiation proceeds until by the twentieth day only a part of the head of the humerus remains. During the same period the muscles and other tissues of the limb undergo dedifferentiation which is just as extreme as that seen in the humerus.

In the second set of experiments, larvae were allowed to regenerate normally for 9 days after amputation of the fore limbs. On the ninth day, when a well-defined blastema had formed, these larvae were placed in a 1: 1000 solution of colchicine for periods of 13 and 24 days. The further differentiation of the blastema was prevented by the colchicine treatment in every case. Furthermore, the limb tissues did not undergo excessive dedifferentiation. The conclusions of Butler and Puckett ('40) are confirmed by these experiments, inasmuch as they show that the excessive dedifferentiation of the colchicine treated limbs was due to the suppression of blastema formation. Colchicine treatment after blastema formation failed to bring about excessive dedifferentiation, because the blastema had already checked dedifferentiation by the time the colchicine treatment was begun.

152. The chromosomes of the alligator. Paul L. Risley, State University of Iowa.

Two-year-old male and female alligators (A. mississipiensis) were injected with powered extract (Fevold method) of whole sheep pituitary glands for varying periods. The longest treatment was for 120 days from May to August, consisting of a total of 3475 mg., or 1390 r.u. Testes, brought into full spermatogenesis by this treatment were preserved in Bouin's as well as osmic acid fixatives (Flemming's and Champy's fluids and modifications).

The diploid number of chromosomes is thirty-two (32) in spermatogonial metaphase figures. One large pair of V-shaped chromosomes is especially prominent, and four additional medium to smaller V-shaped pairs are clearly recognizable. In primary spermatocyte divisions, sixteen (16) pairs are regularly present. No atypical behavior of chromosome pairs justifies the identification of a sex-chromosome pair. In pituitary-stimulated ovaries, mitotic figures are not numerous enough in the material examined to permit an adequate check upon the female chromosome number and pattern.

The centrioles of the spermatogonial prophases and mitotic spindles are long rods, united to form a V-shaped diplosome, as described in turtle spermatogenesis (Risley, '36).

153. The early development of the gonadal rudiment in the opossum (Didelphys virginiana). Olin E. Nelsen and Elisabeth Swain, Department of Zoology, University of Pennsylvania, and E. B. Morris Farm of The Wistar Institute.

The gonadal rudiment makes its appearance during the late tenth day and early eleventh day of uterine development as a thickening of the peritoneal epithelium along the mesial aspect of the urogenital fold. By the late eleventh day and early twelfth day it is a well defined genital ridge consisting of germinal epithelium and contained mass of epithelial tissue.

Differentiation of the testis begins during the first day and is well marked by the second day after birth. There are some features at birth which indicate that the rudiment is already beginning its differentiation. Definite differentiation of the ovary begins during the third day and is continued to the fifth or sixth day following birth. There are, however, certain indications of differentiation as early as the second day. By the end of the first and the beginning of the second week one can be certain of the sex of the young.

154. Variation in rabbit embryos. Allyn J. Waterman, Williams C

Great variation exists in the weight, size and development of em of the albino Flemish Giant strain of rabbit between members of the s r and litters of similar gestation ages. These variations persist throug tation. When plotted against gestation age the smoothed curves show that weight nor size are closely correlated with or are reliable guides to a v may be indicative, depending upon the standard selected for comparis com- bined with data on development of external form and organogen found that development proceeds with regularity when correlated with best

single guide to state of development is chronological age from the moment of mating. Factors responsible for this variability have been sought. They appear in the earliest stages of development and include: state of maturity of ovarian ovum at time of mating; different times after mating when oogenesis is initiated (4–11 hours), ovulation occurs (9–14 hours), and fertilization takes place; rate of development of the follicles; time of implantation; and probably the uterine environment. Mating initiates oogenesis in many ova in one cycle, only a few of which ever ovulate. A 9-day ovary may contain ova in practically all stages of oogenesis. Oogenesis may be accelerated in atretic follicles. In a total of 39 does the average was 8.1 embryos per litter. In the case of the youngest embryos, the cranio-caudal axis may be parallel or at any angle to the longitudinal axis of the uterus.

Endocrinology

155. The control of metamorphosis in Hymenoptera. R. G. Schmieder, University of Pennsylvania.

In two species of Trypoxylon, the results of placing ligatures at the neck showed that pupation and imaginal differentiation depend on a stimulus emanating from the head of the larva during a brief critical period that lies between the fourth and the ninth day of incubation after hibernation. The effects of the injection of critical-period blood into ligated hind ends indicate that a hormone is involved.

In both species, extirpation of the corpora allata before the critical period did not prevent development.

In T. clavatum, the smaller species, extirpation of the brain before the critical period entirely prevented development. Implantation of brains into decapitated larvae was followed by pupation in ten out of fifteen cases.

In larvae of the larger species, T. politum, de-brained before incubation, two out of eleven did undergo development. Of decapitated larvae with implanted brains, only three out of nine underwent development. Attempts were made to secure development by implanting tissues of the head other than the brain. Thus, the entire oesophagus with adjacent aorta, tracheae, corpora allata and, very probably but not identifiable, the corpora cardiaca was used. Two out of seventeen larvae with such an implant showed development. Until we have some explanation for the results in T. politum or until additional experiments are carried out, we cannot be positive that it is the brain in Trypoxylon that supplies the hormone. If not in the brain, the source must be very close to it and thus suffer injury during the usual extirpation procedure.

156. Hormonal regulation of molting and some related phenomena in the crayfish, Cambarus immunis. Harold H. Scudamore, Northwestern University. (Introduced by F. A. Brown, Jr.)

Experiments performed independently of those of Kyer ('42) confirm and extend his observations that eyestalk removal in the winter resulted in gastrolith formation and that sinus gland implants retarded this process which precedes molt. Central nervous system extracts increased the rate of gastrolith formation, suggesting a molt-accelerating action. Mature crayfish normally molt twice a year, spring and summer. Bilateral eyestalk extirpation resulted in molting of 41%

(the remainder died before molt with gastroliths already formed) of the animals observed during the winter, whereas less than 1% of normal animals molted. Gastroliths began to form within 48 hours after eyestalk removal but did not increase greatly in size during the first 7–10 days, but then increased at a rapidly accelerating rate up to molt about 7 days later. The average pre-molt period was 16.26 ± 0.68 days; eyestalkless crayfish formed a second set of gastroliths after the first molt. The gastroliths consisted of a series of concentric layers of calcium compounds, which were found to be deposited in a 24-hour rhythm with active secretion occurring nocturnally.

The "viability effect" of the sinus glands was further established and seemed to be due to something other than simple mechanical difficulty in shedding the exuvium. Carapace measurements of a few crayfish before and after molt revealed a much greater increase in length (growth) in eyestalkless crayfish (5.61%) than in normal animals (1.80%) or eyestalkless animals with sinus gland implants (1.55%) which were observed concurrently. This suggested a growth-retarding principle of the sinus gland.

157. Influence of the sinus glands upon certain metabolic changes associated with molting in the crayfish, Cambarus immunis. Harold H. Scudamore, Northwestern University. (Introduced by F. A. Brown, Jr.)

In normal crayfish there is an increase in rate of oxygen consumption preceding molt. It was found that bilateral extirpation of the eyestalks resulted in increases in oxygen consumption of 50 to 100% and bilateral sinus gland removal, increases of about 25%. Unilateral eyestalk extirpation produced no significant changes. (Cautery of the eye stubs of eyestalkless crayfish resulted in marked increases.) Normal and antennaless crayfishes and ones with severed ventral nerve cord showed no significant changes. The injection of saline sinus gland extracts into eyestalkless crayfish resulted in a decrease in rate to near the normal level. Muscle extracts produced no significant change. Extracts of central nervous organs resulted in increases of 35 to 60%.

Normally, there is a gradual increase in water content for about a week before molt. Bilateral eyestalk extirpation resulted in an increase of 1 to 7% in weight due to increase in water content, whereas normal animals showed decreases as great as 1%. Eyestalkless animals with sinus gland implants increased less than 1%. Preliminary histological studies revealed changes in the kidney epithelium of the eyestalkless crayfish.

There was no detectable difference between the inorganic content of molted carapaces from normal and eyestalkless crayfish. However, eyestalk removal retarded the rate of deposition of soluble lime salts in the new cuticle.

Thus the sinus glands seem to have an influence in regulating respiratory, water, and calcium metabolism, although the specific influences have not been separated from the general molt-inhibiting action.

158. Hormonal influence on molting and the sexual cycle in the crayfish, Cambarus immunis. Harold H. Scudamore, Northwestern University. (Introduced by F. A. Brown, Jr.)

Following the spring molt, most of the male crayfish become form II (sexually non-functional gonopods) and change to form I (sexually functional gonopods)

following the summer molt, remaining in this latter form all winter. Male crayfish, which were induced to molt during the winter by bilateral eyestalk extirpation, changed to form II regardless of previous form. The time of change to form I parallels the peak in testis size and spermatogenic activity reported by Fasten ('14), suggesting the possibility that a male sex hormone may be involved in the appearance of these secondary sex characteristics.

In egg-carrying females there is some mechanism by which the spring molt is delayed until after the young leave the pleopods. It has been shown that removal of the eggs in the spring results in earlier onset of molt. Bilateral extirpation of the eyestalks of egg-carrying females during the winter resulted in molting or gastrolith formation (indicating an approaching molt) regardless of whether the eggs were removed or not. Thus the sinus glands seem to be the source of the molt-inhibitor normally involved. The controlling mechanism of the gland was not determined.

159. Vitamin B_1 and metamorphosis in Rana. E. D. Goldsmith, College of the City of New York, and Washington Square College of Arts and Sciences, New York University, and R. F. Nigrelli, New York Aquarium.

I. Rana pipiens larvae obtained by induced ovulation and fertilization (R. Rugh) were reared in tap water and fed boiled spinach. Tadpoles with hind limbs 7–9 and 10–12 mm. in length were selected. Those in each range were divided at random into four groups: (1) starve; (2) Starve and Vitamin B_1; (3) Spinach; (4) Spinach and B_1. The animals were kept in 500 cc. of tap water, ten in each dish. Group 1 received no food; group 2, no food except 100 mg. of vitamin B_1 dissolved in the water; group 3, spinach daily; group 4, 100 mg. of vitamin B_1 in addition to spinach. All water was changed daily at which time boiled spinach and B_1 were placed in the dishes.

The data (380 animals) show no significant difference in metamorphosis time (forelimb appearance) between the non-treated and the B_1 treated animals. Tadpoles whose hind limbs measured less than 9 mm. when starvation was begun did not metamorphose.

II. Rana sylvatica collected in tail bud stage were reared in tap water. Tadpoles with $2\frac{1}{2}$–$3\frac{1}{2}$ mm. hind limbs were selected and divided at random into three groups. Individuals in groups 1 and 2 received 7 injections of 0.001 cc. of thyrotropic extract (in 0.05 cc. of distilled water). Injections were given on alternate days (with aid of Dr. A. S. Gordon). Vitamin B_1 (200 mg. in 1000 cc. of water) was placed in the dishes of group 2. Animals in group 3 served as controls receiving injections of 0.05 cc. of distilled water on alternate days. None of the controls metamorphosed. Fewer (48%) of the B_1 treated animals metamorphosed than did those receiving only thyrotropin (63%).

Further work is under way.

160. Some effects of testosterone on the sexual pigmentation and other sex characters of the cricket frog (Acris gryllus). B. Greenberg, State University of Iowa. (Introduced by Paul L. Risley.)

Forty female Acris gryllus, implanted with small pellets of testosterone propionate (Perandren, Ciba), all developed the yellow coloration and darkening of

the throat seen in the breeding male. Fifteen males similarly treated before the breeding season uniformly became pigmented while controls remained inactive. Some growth of the vocal sac of winter males resulted from testosterone treatment but they did not call. Males treated during May were occasionally heard to call while controls remained silent. The gonads of treated animals differed little from those of controls. Captivity depressed the gonads of both sexes, when compared with freshly collected specimens. The gonoducts were hypertrophied by testosterone treatment. Wolffian ducts of the female being least affected. Oviducts were strikingly enlarged as also were the seminal vesicles of the males.

Courtship behavior of male Acris collected during May is described. The calls of the males not only stimulate others to call but also incite other males to clasp the nearest individual or pair regardless of sex. A clasped male is released when he vibrates his sides and throat. One function of the call has been considered to be the attraction of sexually receptive females to the males. However, the possibility is suggested that the pigmentation of the throat likewise may attract the females from a distance since the display is very conspicuous when accompanied by vibration of the vocal sac.

161. Hormonal determination of sex behavior in normal and castrate second year juvenile herring gulls (Larus argentatus). Willis R. Boss and Emil Witschi, State University of Iowa.

Reactions of first year herring gulls to sex hormone administrations were described a year ago (Boss and Witschi, '41). The experiments have been continued with twenty-eight birds of the same colony during the second year period. Four treated and three control males were castrated at the age of 1 year. During the second year, normal and castrate control birds remain in the juvenal condition, as attained at the end of the first year. This applies to plumage as well as to voice and behavior. The body weight remains unchanged. There is no difference between normal males and castrates except that the plumage of the former becomes somewhat lighter during the year.

As during the first year period, testosterone propionate (5 mg. per week) causes the assumption and maintenance of the adult plumage. Discontinuation of injections is followed by regeneration of juvenal feathers and resumption of complete juvenal plumage during the molting season. All this is true for castrates as well as for normal males and females.

Voice and behavior reactions differ according to sex. Normal and castrate males assume the various expressive adult calls, but revert to the monotonous juvenal voices after discontinuation of testosterone injections. The male sex behavior, interest in females, aggressiveness and mating preliminaries develop and subside concurrently with the voice changes. The castrates become distinctly more active than normal males. Voice and behavior of testosterone injected females are little different from controls.

Injection of gynogenic hormones produces slight if any effect on plumage, voice or behavior.

162. The influence of some external factors on the ovarian cycle of the female starling. J. Wendell Burger, Trinity College (Conn.).

While increased day-lengths can induce complete spermatogenesis in the male starling, they are not similarily effective in the female. Numerous experiments secured the following data: (1) Added illumination produced in isolated females or groups of caged females oocytes with diameters up to 3 mm. Normally at ovulation oocytes have diameters of 13–15 mm. (2) Added illumination on groups of males and females caged together produced oocytes with diameters up to 5 mm. (rarely 7 mm.). (3) Added illumination on caged pairs (male and female) gave oocytes with diameters up to 10 mm. (4) Females isolated from the wild during the egg-laying season, will lay eggs in laboratory cages. (5) Nest building per se is not a factor. (6) Groups of starlings (3 or 4 pairs each) released in a cage of 4800 cubic feet did not pair. Sperm developed but oocytes reached diameters only up to 4 mm. These data indicate that while increased day-lengths initiate oocytic development, they can not support development of the mature oocyte. The presence of the male and the pairing of the sexes augments the initial light-induced activation. The more intimate the pairing, the greater the oocytic development. Failure to complete oogenesis in cages, may be due to an inability to have normal courtship and ultimate copulation. Experiments giving light to wild birds at their winter roosts is impossible because of blackouts. The author, therefore, takes the liberty to report on a partially completed problem.

163. Ovarian hormones and spontaneous running activity in the female albino rat. William C. Young and William R. Fish, Yale University and The Yerkes Laboratory of Primate Biology.

The importance of progesterone for the induction of copulatory behavior in guinea pigs and rats suggested that the action of ovarian hormones in restoring the level of spontaneous activity displayed by rats prior to ovariectomy should be reinvestigated.

Series A. 10, 40 or 200 R.U. estradiol benzoate in single injections (44 tests) or followed by 0.2 or 0.4 I.U. progesterone (44 tests) were ineffective. Injections of 10 R.U. estradiol benzoate at 4-day intervals for 24 days were ineffective (11 tests).

Series B. 10 or 40 I.U. aqueous estrone daily for 19 days was much more effective, but after about 12 days a progressive decrease in activity began in most animals (14 tests). Supplementary injections of progesterone were without visible effect (12 tests).

Series C. Nearly normal activity followed implantation of 3-mg. estrone pellets (13 tests).

In no animal in which cyclic activity was absent before ovariectomy was treatment effective. Females given daily injections of estrone or pellets displayed cyclic activity simulating that of intact females.

It is clear that the best results depended on a constant source of estrone and that the hormonal control of spontaneous activity is not similar to that of the mating response. The eventual decrease in activity following daily injections is

attributed to irritating effects of frequent treatment. The low effectiveness of injected estradiol benzoate, the activity cycles in the presence of a constant source of hormone and the refractoriness of certain animals to large as well as small quantities cannot be explained.

164. Variations in the sexual behavior pattern in the female guinea pig and rat. William C. Young, Yale University and The Yerkes Laboratory of Primate Biology.

Data from several sources bearing on sexual behavior are summarized and interpreted:

Three types of behavior are conspicuous during estrus in the guinea pig and rat. Both show the copulatory response (lordosis) following manual stimulation or stimulation by the male. Females of both species display male-like mounting activity, guinea pigs more commonly than rats. Individuals of both species show an increase in spontaneous running activity, the measurement of which has been largely confined to the rat.

Lordosis can be evoked in both species by the administration of estrogen followed by progesterone. Refractory individuals respond to increased quantities of estrogen. Mounting behavior in the guinea pig is also shown following administration of these hormones, but in neither intact animals nor in injected spayed animals does any relationship exist between the amount of this behavior and the length of heat. Furthermore refractory animals have not responded to increased quantities of estrogen or progesterone.

Cyclic spontaneous running activity has been induced in rats by the administration of estrogen, but a constant supply of a rapidly utilizable form seems essential, the supplementary action of progesterone is unnecessary and refractory individuals have not responded to increased quantities of estrogen.

In view of these qualitative and quantitative differences in hormonal requirements and the lack of relationship between variation in one part of the pattern with respect to the other parts, the hypothesis is advanced that different mechanisms are involved and that each part of the behavior pattern has its own constitutional or genetic basis.

165. A case of complete hermaphroditism in the grass frog, Rana pipiens. J. Theron Illick, Syracuse University.

Microscopic study revealed the right gonad to consist of testicular tissue only and the left gonad, ovarian tissue only. The oviduct was equally well developed on each side. Of the secondary sexual characteristics, the nuptial pads were poorly developed, in fact they resembled those of a female. Small vocal sacs were present on each side.

166. Testicular response in the English sparrow, Passer domesticus (Linnaeus), to gonadotropic hormone stimulation. Adolph R. Ringoen and John E. Allison, Department of Zoology, University of Minnesota.

Following stimulation of the quiescent and involuted testes of the English sparrow with pregnant mare serum, gonadin (Cutter Laboratories), daily for

periods of 18 to 35 days, pronounced gonadal hypertrophy was obtained. In 25-day treated birds testicular size was 100 times greater than that of control specimens. Histological study of the experimentally enlarged gonads has revealed a number of interesting conditions: (1) tremendous enlargement of the seminiferous tubules with well developed lumina; (2) germinal epithelium in a state of full gametogenic activity and great numbers of mature sperm within the Sertoli cells; (3) hyperplasia of the interstitial elements (previously reported by Pfeiffer and Kirschbaum, '40). Further studies are under way on the diurnal spermatogenic cycle of the experimental birds. According to Riley ('36), "even in the presence of large quantities of an effective gonadotropic hormone, spermatogenic activity awaits the favorable physiological conditions of the dark period."

167. Ambisexual nature of ovarian grafts in female rats (of prostate strain and non-prostate strain). Thomas Hernandez, State University of Iowa. (Introduced by Emil Witschi.)

Autoplastic ovarian grafts were made to ear, leg and tail regions of 210 females. One hundred ten had ventral prostatic lobes and belonged to the prostate strain (PS) while 100 belonged to the non-prostate strain (NPS). Ages at grafting varied from 1 to 173 days. Daily smear records were kept until animals were preserved for histological study at various stages 75–350 days after operation.

If good ear and leg grafts became established before sexual maturity a constant estrus condition appeared earlier than following grafting at adult stages. Females with grafts made prepubertally to the tail region developed the constant estrus condition as they attained sexual maturity.

Pituitaries of constant estrus females average 30% heavier than those of irregular cycle females and 59% heavier than those of controls. The adrenals are increased about 20% in weight over those of control females and 35% over the irregular cycle females.

Eight of the twenty-one prostate strain females with well developed grafts in the tail showed stimulation and two possible stimulation of the prostate. Only four of seventy-two PS females with good ear or leg grafts showed activation and three possible activation of the prostate; in no case was the stimulation as extreme as in some animals with tail grafts. The greatest hypertrophy of the clitoris occurred in constant estrus females with stimulated prostates.

Morphologically the androgenic grafts cannot be distinguished from some purely gynogenic ones. No close correlation between androgenicity and degree of luteinization of the grafts was observed.

168. Ovulation induced in Rana pipiens by bird pituitary preparations. Charles W. Creaser and Roman Harkaway, Wayne University.

Pituitary preparations from the following species have been reported by various authors as capable of stimulating ovulation in the frog: Lepisosteus sp., Rana pipiens, Rana catesbeiana, Rana clamitans, Rana sphenocephala, Bufo marinus, and Triturus viridescens. Preparations from the following species have failed even when administered in high dosage: Squalus suckleyi, Cristovomer namaycush, Perca flavescens, Stizostedion vitreum, Necturus maculosus; mouse, sheep, beef, mare (serum), human (pregn. urine). This refractory nature of the response

has led to the discussion of its specificity. Langan has obtained ovulation in the frog by the use of progesterone and testosterone.

Five cases of ovulation in Rana pipiens have been induced by us using chicken pituitary preparations both fresh and acetone dried. Our first case required forty glands, ten implanted each day; the second 100 glands, implanted over a period of 6 days; the third with 100 mg. (seventy-five glands) of acetone dried; the fourth also with 100 mg. of the same; and the fifth with 90 mg. (sixty-five). Effective pituitary preparations have been derived from fish, amphibian, and bird. A high potency in terms of frog units is found for birds as compared to the mammal. It is suggested from these findings that bird pituitary preparations be used in studies designed to increase ovulation in the domestic fowl.

169. Synchronized induction of ovulation and premature oviposition in the domestic fowl. R. M. Fraps, U. S. D. A., Beltsville, Md.

First follicles of clutch sequences are ovulated prematurely by approximately 24 hours in 50 to 60% of hens intravenously injected about 32 hours before time of normally expected ovulation with 0.1 mg. of a luteinizing extract from sheep anterior pituitaries. In hens exhibiting a 1-day pause between clutches, the terminal egg of the preceding clutch is in utero at the time of injection. Practically all such hens ovulating prematurely also laid this terminal egg prematurely by 3 to 6 hours, or at approximately the time of forced ovulation. The approximate synchrony of effect on follicular and oviducal function is brought out by examination of individual records of hens autopsied within an hour following premature lay. The enforced relation between oviposition of the terminal egg of one clutch and ovulation of first follicle of the succeeding clutch sequence approximates the relation between oviposition and ovulation characteristic of normal intra-clutch successions.

Hens failing to lay prematurely by at least 2 hours failed also to ovulate first follicles of oncoming clutch sequences. The consistent lack of effect on either ovulation or oviposition in about half the injected hens, and approximate synchronization of both events in the remaining birds, suggest that under the conditions of these experiments, one becomes dependent upon consummation of the other or that a common stimulus effects each in independence of the other.

170. The occurrence of regression in ovarian follicles of the hen following administration of a luteinizing extract. R. M. Fraps and A. Dury, U. S. D. A., Beltsville, Md.

In connection with studies of the mechanism of ovulation in hens, large numbers of birds were sacrificed to determine exact follicular sequences and condition of individual follicles at known times following injection of a luteinizing extract of sheep anterior pituitary glands.

In general, regression of the presumably ovulable follicle is favored at dosage levels which induce ovulation in less than 100% of injected hens. Within this range of sub-maximal dosages the ovulable follicle of intra-clutch sequences may undergo regression in all hens failing to ovulate. First follicles of clutches may undergo regression in hens failing to ovulate at sub-maximal ovulatory levels, but some follicles not forced by injection at relatively limited prematurities (8–15

hours) subsequently ovulate normally. Injection levels several times that required for induction of ovulation in all injected hens do not cause regression in immediately ovulable follicles, although regression of second and third follicles of ovarian complements may occur.

Identifiable regression was not found in any of the three larger follicles of ovarian complements of fifty-three uninjected hens sacrificed over the period of several months during which the injected hens were autopsied.

In view of the above relations it is possible that certain types of regression, whether imposed experimentally or of natural occurrence, arise out of normally initiated ovulatory processes which fail to culminate in follicular rupture because of inadequate concentration of the ovulation-inducing hormone.

171. Certain effects of gonadotropic hormones on the developing ovary of the opossum. Olin E. Nelsen and Elisabeth Swain. Department of Zoology, University of Pennsylvania, and E. B. Morris Biological Farm of The Wistar Institute.

Treatment consisted of injections of follicle stimulating factor (Ayerst, McKenna and Harrison) followed by a combination of this factor with the luteinizing gonadotropin present in antuitrin-S (Parke, Davis and Co.). Opossum ovaries approximately 4 months old respond with subsequent swelling of certain follicles, luteinization and formation of small ovulation points on the ovarian surface. It is probable that a few eggs were ejected. Five-, 7- and 8-month ovaries respond readily, but ovaries of 3 months and younger fail to respond perceptibly to this treatment.

It is suggested that the oval-shaped follicle roughly averaging 200 μ by 300 μ may be the size whose further development is controlled by pituitary gonadotropic substances. Development of follicles to this approximate size is probably carried on independent of pituitary gonadotropins. It is further suggested that the ovary from a time shortly after birth to sometime between 3 and 4 months is an irrevocably determined, self-differentiating structure unaffected by gonadotropic or sex hormones. One of the main features of this period of ovarian development is the segregation of germ cells and formation of young follicles. While the germinal epithelium is very active during this time in giving origin to germ cells, there is some evidence that this activity is somewhat irregular, periods of activity alternating with periods during which the epithelium is not so active.

172. The effect of estrin on the function of corpora lutea in hypophysectomized rats. Warren O. Nelson and J. Walton Pichett, College of Medicine, Wayne University.

The action of estrin in normal adult female rats includes the maintenance of diestrus for 16–20 days. This appears to be due to the release of some corpus luteum-maintaining factor, possibly luteotrophin, from the hypophysis. The animals eventually return to continuous vaginal estrus due to either an inability of the corpora lutea to continue functional activity or to a depletion of the hypophysial factor.

Westman ('37) and Robson ('37) have presented evidence in the rabbit which they interpret as indicating that estrin acts directly upon corpora lutea in maintaining their function. This has been put to test in the rat.

Forty-six adult female rats were injected with 200 I.U. estrone daily throughout the course of the experiment. This treatment uniformly induced diestrus. Ten animals left intact returned to estrus after 16 to 20 days. Fourteen rats were spayed on the fourth, seventh or tenth day after the initiation of treatment. In each case the animal returned to estrus on the third or fourth day after castration. Twenty-two rats were hypophysectomized on the fourth, seventh, tenth, twelfth or fourteenth day after initiation of treatment. Estrus returned in each case on the third or fourth day after hypophysectomy, an exact parallel with the result in spayed animals.

These results indicate that estrin has no direct effect upon corpora lutea in the rat, and that its action is exerted by way of the hypophysis.

173. Age changes in the anterior pituitary gland of the mouse. Kathryn F. Stein, Elizabeth Caldwell and Jean Peters, Department of Zoology, Mount Holyoke College. (Introduced by Christianna Smith.)

Pituitaries of mice show definite differences due to age. The percentage of eosinophiles is greatest in newborn animals and lowest in senile females (2 years or over). The males show a fairly constant percentage at 25 and 60 days with a decrease in old age, in females there is a significantly lower percentage at 25 days, and a greater decrease than in males due to old age. The peak for the basophiles occurs in females at 25 days. At all other ages studied the male percentage is higher. Basophiles seem to increase in size with increasing age. Other cells are larger during adult life than in newborn animals, but decrease with senility. Chromophobes are more numerous than eosinophiles or basophiles at all ages, with the possible exception of newborn animals. They are especially numerous in senile females. The differentiation of cell types becomes more distinct with increasing age. Vacuolated cells and cells with solidly staining nuclei are most numerous in old animals, and the latter are especially characteristic of chromophobes. Glands of senile animals fixed in Champy show lipoidal droplets in the cells, and smaller Golgi networks in all except the basophiles. The reticular framework increases in extent and amount of branching with age. Mitotic divisions were noted only in the 25-day-old glands and were usually found in chromophobes. Some of the histological differences observed at different ages can be correlated with variations in physiological activity.

174. The effects of thyroxin administration on the adrenals, thyroids and anterior pituitaries of non-pregnant and pregnant mice. A. Elizabeth Adams, Mary Medlicott and Marjorie Hopkins, Mount Holyoke College.

Total doses of thyroxin (0.6 mg. or 1.2 mg.) administered in 6 days to non-pregnant and pregnant (beginning 12½ to 14 days post-copulation) albino mice induced changes in the adrenals, thyroids and anterior pituitaries of the hosts compared with uninjected controls. The adrenals in both groups increased in weight, and in both the enlargement was due to hypertrophy of the cells of the inner fasciculata of the cortex and, in case the X zone was still present (as in virgin females) or involuting (as in primiparous ones) or represented by a few cells (as often occurs even after a first pregnancy), to hypertrophy of its cells. The thyroids and anterior pituitaries of the thyroxin-injected non-pregnant mice

were smaller and appeared less active when examined histologically than those of untreated controls, since the thyroid epithelium was lower and the basophiles of the anterior pituitary were more granulated as if in a storage phase and the acidophiles degranulated. In the control pregnant mice, thyroids and pituitaries were larger and more active histologically as evidenced by higher thyroid epithelium and more vacuolated thyroid colloid and enlarged and degranulating acidophiles and basophiles in the pituitary compared with the condition in non-pregnant ones. In thyroid-injected pregnant mice, there appeared a lessening of the hyperactivity of thyroid and pituitary seen in untreated pregnant animals but it did not reach the level of hypoactivity of the thyroxin-injected non-pregnant group.

175. Factors influencing antihormone production. Albert S. Gordon, Department of Biology, Washington Square College of Arts and Science, New York University.

It has been shown (Gordon, '41) that purified pregnant mare serum (PMS) preparations evoke more antihormone (AH) in rabbits than crude samples. Two possible explanations for this phenomenon are that either some factor exists normally in blood serum which inhibits the AH response or that some substance is produced in pregnant animals which interferes with AH formation.

To test these possibilities, six groups of rabbits were injected daily, for 2- and 5-month period, with 20 Cole-Saunders (C-S) units of purified PMS. Group A received, in addition, daily injections of 2 cc. whole normal horse serum; group B, 1 cc. concentrated horse blood globulin solution; group C, 1 cc. concentrated horse blood albumin solution; group D, 40–80 R.U. estradiol; group E, 1 cc. 1–5000 merthiolate. Group F received hormone only. The antihormone content of the sera was assayed in immature female rats. Albumin was the only substance found to inhibit antihormone production, and its effect was not marked.

In another experiment, rats pregnant for 12–19 days were injected daily with 5 C-S units of PMS for 25 days. Injections given to rats earlier in pregnancy resulted in the death and resorption of fetuses. Similar experiments were conducted with pregnant guinea pigs injected for 2 months. The sera of the injected pregnant animals and animals in which fetuses were being resorbed contained considerably less AH than sera from injected normal animals.

It is concluded that normal serum proteins do not influence materially AH production to PMS. Some factor, however, appears in pregnant (and probably lactating) animals which inhibits AH formation to injected PMS.

176. A preliminary study of changes in the blood of gray Norway rats following audiogenic seizures.[1] William H. Cole, Eleanor H. Yeakel and Edmond J. Farris, Rutgers University and The Wistar Institute of Anatomy and Biology.

Determinations of pH, CO_2, non-protein nitrogen, potassium, phosphate, and clotting time were made upon blood of gray rats after audiogenic seizures. Experimental animals were subjected to the sound of an air-blast 5 minutes daily for 5 days a week since weaning, in an investigation of the effect of neurophysiological stimuli upon life processes in the rat.

Air-blasting precipitated violent, aimless running followed by epileptiform convulsions. During the subsequent stage of semicomatose passivity, a sample of blood was drawn from the heart. Plasma filtrates were pooled for four complete determinations. Blood for clotting tests was obtained from the tail in a parallel series of rats.

The pH of the blood from the experimental rats dropped sufficiently in all cases to indicate an acidosis, the average from four determinations being 6.96 (control, 7.40). The CO_2 content of whole blood also decreased significantly, from 14.5 millimoles per liter to 8.1. The potassium and phosphorus contents of the plasma from experimental animals did not show significant differences from those of normal rats. The non-protein nitrogen values were slightly lower than those from normal rats but not especially significant. Clotting time was reduced from 114 seconds to 35.

Although the number of determinations was small, the results showed that, following an audiogenic seizure, the gray Norway rat rapidly developed a shorter blood coagulation time, and an acidosis caused by acids other than carbonic in the blood.

[1] This investigation was aided in part by a grant from the Samuel S. Fels Fund and the M. J. Greenman Research Fund.

177. Effect of feeding two eggs from a colchicine injected hen to 1-day-old baby chicks. Edna Higbee, The University of Pittsburgh.

(No abstract submitted.)

General Physiology

178. Variable factors of fertility in Lymnaea columella. Grace Springer Forbes, Barnard College, Columbia University. (Introduced by Henry E. Crampton.)

In Lymnaea columella an analysis of fertility was made using as the fundamental data, age at onset, length of the fertile period, age at death, number of egg masses laid, average size of the egg masses, and the total number of eggs produced. An attempt was made to derive, from the more easily obtained variables, a theoretical value which could be used to express the total productivity of a family of snails; and an analysis of the number of egg masses laid in relation to the total number of eggs produced was carried out as an integral part of the problem to determine whether egg production represents essentially one simple process or a more complicated bi-variant physiological function of the organism.

It was concluded that the total productivity of a family of snails can be accurately described to the satisfaction of this investigator only in terms of the actual number of eggs produced. The number of egg masses produced may not be used as the index of fertility, presumably because more than one bio-chemical process is at work in egg production in Lymnaea columella. Corroborative evidence is presented for this hypothesis.

There is no evidence that age at onset or age at death has any direct relation to the reproductive process.

179. The effect of added amounts of vitamin B_1 on the life cycle of Lymnaea columella. Grace Springer Forbes, Barnard College, Columbia University. (Introduced by Henry E. Crampton.)

Small amounts of vitamin B_1 added to the standard diet of Lymnaea columella exert a stimulating effect on growth and fertility, while larger amounts retard these functions of the organism. There is some evidence that the life cycle is significantly shortened in both instances. The suggestion is offered that these results may be due to dietary imbalance and work to ascertain the truth of this hypothesis is under way.

180. The digestion of collagen by enzymes. Irwin W. Sizer, Massachusetts Institute of Technology.

Collagen has long been characterized by histologists and histochemists by its ready digestion by pepsin and by its resistance to attack by trypsin. Thus, trypsin is commonly used to remove many non-collagenous components from connective tissue. The present investigation indicates, however, that pulverized collagen (no. 20 mesh) separated from skin or tendon of beef or rat is readily digested by trypsin at 37° over the pH range from 6 to 8. Digestion of the collagen suspension was studied by measuring the decrease in weight of the insoluble material, the increase in weight of soluble material, the increase in soluble nitrogen and the increase in formol titration of the solution which occurred during the course of digestion. All methods used indicated that trypsin (both crude and crystalline) and similar enzymes such as orthozyme, papain, and keralin, produced a marked hydrolysis of suspensions of pulverized collagen in various stages of purification obtained from different sources. Approximately 70% of the insoluble collagen was dissolved in 18 hours, although most of the action occurred in the first 5 hours. The undissolved residue can be further digested if it is dried and again pulverized and resuspended in a trypsin solution. The rate of digestion seems to be roughly inversely proportional to the size of the particle of crude tendon or skin or highly purified collagen to be digested. The difference in the action of trypsin on collagen in tissues and in powder form may be related to the size of the fibrils, or to the presence of a trypsin-resistant coating encasing the collagen in the tissues.

181. Islets of Langerhans in the pancreas of white rats. Walter N. Hess, Hamilton College.

Studies of the islets in the pancreas of the Wistar strain of white rats shows that factors which increase the rate of metabolism cause an increase in the number of islets together with an increase in islet tissue. Such factors include exercise, the injection of anterior pituitary extracts, and the feeding of desiccated thyroid gland substance. An increase in islets and islet tissue is also caused by factors that cause an increase in blood sugar such as the feeding of large amounts of glucose, the injection of adrenalin, and especially the intraperitoneal injection of glucose. Factors that lower the rate of metabolism such as the removal of the thyroid glands appear to cause a reduction of islets and islet tissue. It was also found that the addition of cholesterol, and of lecithin to the diet caused an

increase in islets, while the injection of protamine insulin resulted in a reduction of islets and of islet tissue.

None of the above factors influence materially the amount of acinar tissue in the pancreas. However, the daily injection of pilocarpin for a period of 4 weeks causes a great increase in the weight of the pancreas without increasing the islets and islet tissue.

It is concluded that the islets have great power of adjusting to differences in the metabolic rate as well as to differences in the blood sugar level, and that the acinar tissue of the pancreas adjusts readily to demands for the exocrine secretion.

182. The production and analysis of feather-chimaerae in fowl. Frank R. Lillie and Hsi Wang, Whitman Laboratory of Experimental Zoology, The University of Chicago.

Autoplastic combinations of dorsal and ventral halves of breast (B) and saddle (S) papillae of regenerating feathers of brown Leghorn capons were made in follicles of the host component. From such unions feather-chimaerae exhibiting characters of both tracts develop and persist in successive regenerations. Symmetrical B/S combinations produce longitudinal (''marginal'') chimaerae, B in center and S at margins. Symmetrical S/B combinations produce transverse chimaerae the apex of which is S and the base B with a transitional zone between composed serially of barbs with increasing B apexes and diminishing S bases. Asymmetrical combinations produce asymmetrical pseudobilateral chimaerae of two kinds. Occasionally a single combination produces twin feathers, each of which is a chimaera. The B/S composition of the collar is stable throughout development; hence apexes of barbs form from ventral S cells, and bases from the dorsal B cells. The S/B composition of the collar is unstable, the ventral B cells replacing the dorsal S cells gradually but completely in the direction of tangential movements of barbs. Although S cells cannot replace B cells in the collar, they act on them in the direction of tangential movement and inhibit the oxidation of the melanin precursor, so that B melanophores adjacent to S cells remain brown and never reach the black stage. The lengths and fluff-ratios of feather-chimaerae are intermediate in relation to B and S feathers. Growth rates are determined by the dorsal component, in which the rhachis seems to act as pace-setter for both components. These results fit into the theory of the mechanism of feather development as previously stated.

183. The morphogenetic role of the dermal component of the papilla, and feather-forming capacity of the general follicular epidermis. Hsi Wang, Whitman Laboratory of Experimental Zoology, The University of Chicago.

The change from donor tract specificity to that of the host in feathers regenerating from reciprocally transplanted Brown Leghorn capon breast (B) and saddle (S) papillae, denuded of their epidermal covering,[1] demonstrated that tract specificity including pigmentation is determined exclusively by the epidermal component of the papilla. When similarly denuded S papillae were transplanted

[1] Wang, H. 1941. Anat. Rec., 81 (no. 4 suppl.): 40.

to B follicles with their dorsal and ventral surfaces reversed by rotation, none of the resulting feathers displayed normal orientation; most of them were inverted dorso-ventrally. This confirms the conclusion reached earlier [2] by using a different technique that the seat of determination of symmetry is inherent in the dermal papilla alone. That a papill-deprived follicle when grafted with a denuded papilla from either the same or different tract resumes feather regeneration suggests that feather-forming capacity is not limited to the regeneration cells (epidermal component). To determine the extent of this capacity, a denuded S papilla was transplanted to the follicular wall of a B follicle at different levels either in the absence of its own papilla or with the latter remaining intact. The grafts yielded B feathers in both cases (in the latter, two feathers growing in the same follicle) up to 4 mm. from the bottom. These results show that the epidermis of at least the basal half of a follicle is equivalent to the regeneration cells in potency when provided with the indispensable morphogenetic influence of a dermal papilla. Denuded papillae transplanted to extra-follicular (skin) epidermis above the breast, however, gave negative results.

[2] Lillie, F. R., and H. Wang. 1941. Physiol. Zool., 14: 103–133.

184. Observations on the light requirements of the Weaver finch, Pyromelana franciscana. Marie Rollo and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.

Male weaver finches in eclipse plumage were found to respond to an increased daily light period by assuming nuptial plumage. We were interested in determining the optimum period and intensity required to produce this plumage.

A rack divided into 20 by 20 inches, light-proof compartments was used for experiments on optimum period. Nine males were put into each compartment. They were given daily light periods varying from 10 to 18 hours. Arrangements were regulated so that they were exposed to 10 hours of daylight with all additional light supplied from a 100-watt bulb suspended from ceiling of each compartment. Birds on 14-hour period were first to respond by a change in plumage and were first to complete their molt. The response of the others was in direct proportion to length of period above and below the optimum.

For observations on intensity the rack was placed into a darkened room where birds in eclipse daily received 14 hours of artificial light. Intensity was measured in foot-candles in three places of compartment and averaged. Intensities from 3½ to 350 foot-candles were provided. We found 126 foot-candles intensity to be the optimum, since birds on it were first to develop nuptial plumage and completed molt 5 to 6 weeks ahead of those on other intensities. At the low and high intensities no eclipse feathers were regenerated.

Weaver finches can be brought into full nuptial plumage in 10 weeks on a 14-hour daily light period at 126 foot-candles intensity. Anything above or below optimum causes regression.

185. The carotenoids of the display plumages of African bishop birds. Henry Kritzler, State University of Iowa. (Introduced by Emil Witschi.)

Analysis of the scarlet feathers of the display plumage of Euplectes franciscanus, E. orix and E. nigroventris collected in the natural habitat shows presence of three

carotenoids. In order of decreasing adsorbability, they are a red pigment R_1, yellow lutein, and red pigment R_2. The last one is consistently missing in captive specimens, which accounts for the more yellowish color of captive males.

The yellow feathers of the canary and of three species of yellow weaver and bishop birds, contain a mixture of two pigments: lutein and a red pigment resembling R_1, without being identical with it.

Analysis of the diet of the captive birds failed to show the presence of a carotenoid which could be identified with either one of the red pigments, suggesting that R_1 may be an intermediate in the oxidative decomposition of dietary carotenoids. The red substance R_2 is possibly a derivative of lycopene, which assumption is supported by the fact that tomato-fed franciscanus and nigroventris males regenerate display feathers containing R_2. Apparently the standard diets of captive birds are deficient in lycopene or a closely related substance. The source — vegetal or animal — from which wild birds get this base material for the R_2 pigment is not yet known.

The failure of tomato feeding to affect the yellow feathered species suggests that these are unable to absorb polyene hydrocarbons from the intestine.

Capsanthin appears in the plumage of paprika fed bishop birds, but it is not found in the feathers of wild birds.

186. Relation of thyroid gland to plumage pattern and gonad function in the brown Leghorn male. Ben Blivaiss and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.

Thirty adult cases of complete thyroidectomy were obtained from a large series of brown Leghorn cockerels which had undergone thyroid removals on the fourth to twentieth day after hatching. Crop-binding and respiratory disturbances which commonly developed post-operatively were frequently fatal.

Thyroidectomy delays the appearance of definitive feathers, juvenile feathers in all regions persisting longer than normal. Juvenile feathers, especially on the neck, ventral and lateral body areas show a decrease in melanin and barbules and an increase in red pigment.

In the adult thyroidectomized rooster, body contour feathers show a loss of melanin and accompanying sheen which is replaced by a red pigment. They also show a decrease in the number of barbules resulting in a hackled, fluffy appearance and a tendency to become elongated, narrow and tapered. Some feathers not only show a decrease in barbules but also a reduction of hooks. Certain of the barbs have malformed barbules which lie nearly parallel to the barbs instead of extending laterally.

Cases of incomplete thyroidectomy show only a partial replacement of melanin with red pigment and little or no reduction in barbulation on the body contour feathers, especially on breast, ventral and lateral surfaces.

Body weights of adult thyroidectomized roosters in our experiments were 40–45% below those of normals. Head furnishings were also smaller, combs averaging 62–68% less than those of controls. At 8 months of age, histological observations indicate a delay in spermatogenesis. The testes have smaller tubules and in no case show development stages beyond spermatocytes.

187. Relation of thyroid gland to plumage pattern and gonad function in the brown Leghorn female. Ben Blivaiss and L. V. Domm, Whitman Laboratory of Experimental Zoology, The University of Chicago.

Thyroid removal was performed on a large series of brown Leghorn pullets from which ten adult cases of complete thyroidectomy were obtained. In the pullets as in cockerels, thyroid removal was performed on the fourth to the twentieth day after hatching in two unilateral operations. Intervals between operations were 3 to 5 days. A majority of the bilateral operations were completed by the tenth day. Post-operative survival, as in males, was seriously hampered by the frequent development of a crop-bound condition due to trauma as well as respiratory disturbances.

Juvenile and mature body contour feathers of a thyroidectomized hen show the same type of modifications as do the feathers of a thyroidectomized rooster. A striking similarity in appearance is observed in breast, ventral and lateral body feathers of the two sexes, being less conspicuous in other feather areas. Juvenile feathers in all body areas are narrow, red and fluffy in appearance and have a delayed molt. Adult dorsal and lateral body feathers lose their typical mottled pattern becoming narrow and elongated, red in color, hackled and fluffy in appearance.

Partial replacement of melanin by red pigment with little or no decrease in barbulation occurs in the event of incomplete thyroidectomy of hen or rooster. This would seem to indicate a lower threshold for modification of pigment than for loss of barbules.

Body weights of adult thyroidectomized hens are 27–35% below those of the controls while comb averages are 70–75% smaller. None of our thyroidectomized hens which are now 8 months old have been known to lay.

188. Feather succession in a case of hemi-lateral asymmetry. Mary Juhn, University of Maryland.

A cockerel with asymmetric distribution of plumage characters was first noted while in the juvenile stage; it remained under observation until in process of assuming adult feathering during which time it died. The first plumage was largely barred black and white but with sporadic red feathers or segments on the left and large red areas on the right and frequently showing a clear demarcation along the mid-line.

Comparison of first and second generation feathers from given regions and from specific follicles demonstrated a definite tendency toward the replacement of barred by red. This was more marked on the right, which increased the degree of initial asymmetry. The right leg was of more reddish-cream pigmentation than the left. Both irises were similar in shade. Molt occurred at a later date on the left side and the feathers were somewhat longer. Asymmetry of the appendicular skeleton was very slightly in favor of the left; of the internal organs only the left testis was conspicuously larger than the right.

Identity of the plumage genotype was obvious in the all-over appearance of the component feather traits. The quantitative difference in the distribution of these traits to right and left indicates the participation of a physiological variable beween the body sides. A hemi-lateral differential remains a fixed property but within this frame the intensity of the action varies with age as shown in the feather successions.

189. Feather pigmentation and leucosis in the brown Leghorn. Mary Juhn, University of Maryland.

Brown Leghorn males have been under observation since January 1939 at which time cocks and capons with symptoms of leucosis were found to show characteristic and consistent modification of normal feather pigmentation, black being replaced by red. Successive feather generations were collected from suspect fowls and controls. Autopsies were performed at death. Fowls with aberrant plumage were found with lesions recognized in the avian leucosis complex; to date this same aberrant pigmentation has not been found in birds otherwise diseased nor has the tuberculin test in abnormally feathered fowl given a positive response.

The pigment changes are accordingly considered to be associated with leucosis; when the course of the disease is slow, a definite sequence in the replacement of black by red may be traced in the breast tracts. Here the barb apices of the central vane segment of the lateral feathers are first to be modified.

Diseased fowls show no evidence of hypothyroidism but injection of 5 mg. thyroxine induces return of black pigmentation in modified red feathers as well as melanin and barbulation extension in laced regions, these last two reactions being comparable to similar dosage effects in normal birds.

In interpreting the color changes of the plumage in leucotic Brown Leghorns it is first assumed that black is the oxidized form of red pigment. The deposition of red pigment in the place of black is then considered a reflection of an alteration of the oxidative mechanisms in leucosis and as indicative of the presence of this disease.

190. The effect of thyroidectomy on spermatogenesis in the adult male guinea pig. Landrum B. Shettles and G. E. Seegar Jones, Departments of Obstetrics and Gynecology, the Johns Hopkins University, Baltimore, Maryland.

Thyroidectomy in the lower experimental animals has been shown to have no effect on either the gross or histological appearance of the testis. It was thought that finer morphological changes in the spermatozoa themselves might not be demonstrated by such gross techniques. In the present experiment artificial ejaculation was produced electrically in mature male guinea pigs, and the semen examined microscopically with reference to number, motility, and morphology of the spermatozoa. These animals were then thyroidectomized and after a month of rest were again stimulated electrically at weekly intervals and their semen examined. No changes were noted in the number, motility, or morphology of the spermatozoa following thyroidectomy. As a final check on normal spermatogenesis in the absence of the thyroid gland, the same thyroidectomized animals were mated with females of known fertility. All of the animals in the present series became pregnant and delivered normal litters, with the exception of three pigs, two of which delivered macerated fetuses at 61 and 48 days, respectively; the third pig died on the thirty-fourth day of her pregnancy. Examination of the fetuses showed no abnormalities.

191. The cycle of egg and sperm production in Amphiuma tridactyla Cuvier. Francis H. Wilson, Tulane University.

Collections during the period of April to June 1941 showed that only sixteen of thirty-seven mature females were carrying large eggs suitable for oviposition during the year. Collections for a similar period in 1942 showed that only twenty-six out of a total of fifty-four mature females were carrying large eggs nearly ready for oviposition. This suggests either that only approximately 50% of all mature females are capable of forming layable eggs or that 2 years are required for the ovary to develop ripe eggs. The 50% rule is approximated for each age and size group suggesting the probability that the young do not all mature their first crop of eggs at the same size or age.

The males once they have reached the size or age for sperm production form quantities of sperms yearly since all males collected in 1941 and 1942 except those in the smaller size groups, show evidence of having produced sperms in the early spring.

192. Seasonal changes in the reaction to the turtle's vagus. Dorothy Fetter, Helen C. Coombs, Brooklyn College, and F. H. Pike, Columbia University.

The turtle's heart stops, in winter, on excitation of the right vagus with a low intensity of induced current, but frequently fails to respond at all to the left. The stomach responds to the left vagus by well marked contractions, but frequently fails to respond to the right; and greater intensity of current is necessary than for the heart. Both heart and stomach may fail to respond to excitation of either vagus after introduction of strophanthin into the stomach, but failure of the stomach reaction is constant, and comes on about 90 minutes earlier.

The response of the heart is erratic in spring, frequently failing on excitation of either vagus, while the stomach always responds to the left vagus, and usually to the right.

The response of the heart is more regular in summer. Both vagi become effective, but greater intensity of current is necessary than in winter, in the ratio of about four to one. The stomach responds to either vagus, but only about half as much current is necessary for the left vagus as in winter. Strophanthin abolishes the vagus effect on the stomach, but usually not its effect on the heart. Perfusion with varying concentrations of potassium and strophanthin shows a wide difference in cardiac deportment in summer and in winter.

The quantitative variations in reactions of the heart and stomach appear to be those best adapted to the seasonal variations in activity of the animal.

193. Comparison of the role of the afferent vagal proprioceptive fibers in respiratory control in birds and mammals. W. C. Randall and W. A. Hiestand, Western Reserve University and Purdue University.

That the vagal nerves contain inspiro-inhibitory or expiro-excitatory fibers or both has been indicated by various findings since the early investigations of Hering and Breuer. It is generally acknowledged that the vagi possess a function limiting the sustained inspiration of apneusis. The degree of this activity varies with

birds and mammals. Section of one vagus in the rabbit causes deceleration of the breathing rate while section of the remaining vagus causes a still greater inhibition. Birds after vagotomy are barely able to breathe. The respiratory rate of the domestic fowl after bilateral vagotomy may drop to as few as four strokes per minute with a slow but progressive increase with successive days. These effects are due to the removal of the influence of the afferent vagal stretch fibers resulting in a return toward the primitive apneustic type of breathing of lower forms.

Section of either or both vagi during panting in the mammal has no effect on respiratory rhythmicity which shows that the vagi are unnecessary for mammalian panting. However, in the bird (domestic fowl) section of both vagi abolishes panting with the rhythm returning immediately to or slower than the non-panting rate. If only one vagus is sectioned during panting no change in rate occurs, nor does it matter which vagus is cut so long as one is left intact. Thus it can be concluded that at least one vagus is necessary for panting in birds but not in mammals.

194. Respiration of the Reptilia as influenced by the composition of the inspired air. D. E. Stullken, W. C. Randall and W. A. Hiestand, Purdue University.

The normal respiration of all reptiles used in this study (fourteen species representing the four major orders of the class, Reptilia) is apneustic in nature and consists of an active expiration followed by one or more inspirations and a final slow passive expiration as the lungs return to a normal state of maintained partial distention.

In the turtle carbon dioxide produces an initial apnea which is followed by increase of both rate and amplitude of breathing. Severe hypoxia produces an initial increase of amplitude with some increase of rate, although slight traces of oxygen appear sufficient to maintain life for long periods.

In the snake carbon dioxide produces decreased amplitude with a prominent increase in rate of breathing. Hypoxia produces a progressive increase of amplitude followed by an acceleration in rate.

In lizards prompt inhibition of breathing generally results with administration of low percentages of carbon dioxide. High concentrations of carbon dioxide generally produce definite stimulation. Augmentation of amplitude follows low oxygen administration.

In the alligator carbon dioxide produces definite augmentation of both rate and amplitude, the rate of breathing and volume of air breathed being approximately proportional to the percentage of carbon dioxide inhaled. Nitrogen (fulminating anoxia) causes an immediate and progressive increase in amplitude in the alligator.

Hyperoxia in all reptiles used causes a slight decrease in both amplitude and rate of breathing.

195. The responses to light of various wave lengths by the periwinkle, Littorina litorea (L.). Dwight Elmer Minnich, University of Minnesota.

The periwinkle, Littorina litorea (L.) when extended responds to an appropriate decrease of light intensity by retraction. The extent of response varies with the condition of adaptation, the strength of the stimulus, and other factors. This

response has been studied under carefully controlled conditions to four regions of the spectrum of equal energy content, viz. red, 6500–7400 Å, yellow, 5600–6800 Å, green, 4500–5800 Å, and blue violet, 3800–4850 Å. To red the snails are wholly unresponsive. To yellow, green, and blue violet, however, they respond. By varying the intensity, the thresholds for these three colors were determined and their effectiveness compared. Green was by far the most effective, followed by blue violet and finally yellow. The exposure required for a response to light reduction varies from 1 to 3–4 seconds. By repeated exposures of 10 seconds followed by light reduction, the animal finally becomes unresponsive. The number of repetitions required to effect this state varies, but usually exceeds thirty. Snails rendered unresponsive to one of the colors were tested with the other two. Animals rendered unresponsive to green failed to respond to yellow or to blue violet. Animals rendered unresponsive to yellow or to blue violet failed to respond to the other two regions in more than 90% of the trials. These facts show that in the photoreceptors of this snail there is present but a single photosensitive substance with maximum absorption in the green, and that the animal is, therefore, unable to distinguish different wave lengths of light.

196. Correlation of photochemical events with the action potential of the retina. Verner John Wulff, State University of Iowa. (Introduced by T. L. Jahn.)

Curves demonstrating the intensity-EMF relationships of the action potential were obtained from the eyes of dark-adapted normal grasshoppers (Melanoplus differentialis) and moths (Samia cecropia and S. synthia) and from grasshoppers from which the optic and cerebral ganglia had been removed.

Intensity-EMF curves were also obtained from the eyes of normal and deganglionated grasshoppers which had been light adapted by means of continuous exposure to one per second half-second flashes of light of different intensities. In these experiments the magnitude of the response was measured after an equilibrium had been reached at the given intensity. All of the curves for both the dark-adapted and light-adapted animals were S-shaped.

Calculations of the per cent of the photosensitive material decomposed as a function of intensity under in vitro conditions simulating those of the dark-adapted eye (based on the data of Hecht) also yield S-shaped curves. Similarly, calculations of the amount of photosensitive material decomposed in an eye in a steady state equilibrium of adaptation by light of intensity equal to that of the adapting intensity also yield an S-shaped curve. It is concluded that relationships between EMF and the amount of photosensitive material decomposed is approximately linear under conditions of both light and dark adaptation. Therefore, the agent T in the reaction postulated by Hecht ($L // P + A // \rightarrow T$, where L is an indifferent material, $P + A$ is the decomposed photosensitive material, and T the activating agent) may be the action potential of the retina.

197. Intensity-EMF relations of the electroretinogram of beetles possessing a diurnal rhythm. Verner John Wulff and Theodore Louis Jahn, State University of Iowa.

Intensity-EMF curves have been obtained for Dytiscus and Hydrous during the day phase and during the night phase. Throughout the entire intensity range

studied (0.001 to 10,000 foot-candles) the magnitude of the day-phase response was considerably smaller than that of the night-phase response. These data indicate a higher sensitivity for the night-phase eye, and they are in agreement with previous results which demonstrated almost a thousandfold change in threshold between eyes in the day and night phases for Dytiscus and magnitude differences at high intensity for both Dytiscus and Hydrous.

The intensity-EMF curves for the *b*-wave of Hydrous during both the day and night phases are S-shaped and are similar in form to those for various other animals. In the ERG of Dytiscus it is difficult to detect and impossible to measure the *b*-wave accurately, and curves for the *a*- and *c*-waves of the night phase response are not S-shaped. One reason for this is that the wave form of the ERG varies greatly with intensity and changes from a response which is almost all *a*-wave to one in which the *c*-wave is predominant. Therefore, the use of the magnitude of the *b*-wave as a measure of sensitivity is not feasible in Dytiscus.

198. The effect of temperature on the retinal action potential. Theodore Louis Jahn and Verner John Wulff, State University of Iowa.

The effect of temperature on the magnitude of the electrical action potential in response to 17 and 500 msec. flashes of light of high intensity (10,000 foot-candles) was determined for the light- and dark-adapted photoreceptors of normal and deganglionated grasshoppers (Melanoplus differentialis). Comparison of Q_{10} values for comparable temperature ranges revealed that the values for the light-adapted photoreceptor were consistently higher than those for the dark-adapted photoreceptor. It is suggested that above 15°C. the dark-adapted photoreceptor Q_{10} values (average of 1.25) pertained to the initial photochemical or light reaction which is the master reaction of a catenary series and that below 15° the Q_{10} values (average of 1.9) were those of the potential producing reaction which is inferred to be a thermal catalytic reaction. It is also suggested that the Q_{10} values for the light-adapted photoreceptor (average of 2.0 above and 3.2 below 15°C.) represented the temperature coefficients for the dark reaction which is the master reaction in the light-adapted state. Removal of the optic and cerebral ganglia did not substantially change the above Q_{10} values. This explanation is in agreement with the theory that the magnitude of the retinal action potential in these experiments is a linear function of the amount of photosensitive pigment decomposed.

199. The effects of a sudden decrease in temperature on the blood picture of cattle. Elaine T. Delaune and Roy L. Mayhew, Louisiana State University. (Introduced by Ellinor H. Behre.)

The general picture produced by a sharp drop in temperature was a sharp rise in the total number of leucocytes, no noticeable change in the total erythrocyte and hemoglobin counts, with a sharp rise in the per cent of monocytes and a corresponding decrease in the per cent of lymphocytes. No changes were noted in the other differential leucocyte counts. The normal preinoculation range of leucocytes on thirty-two animals taken over a period of 5 years was 9,500 to 11,000 for most individuals. Sharp thermal drops as great as 37° produced increases as high as 26,750 over the normal individual range.

In order to further substantiate these changes, three normal animals were placed in a heat controlled room and two more normal animals were placed in a room not supplied with heat. During five successive periods of sharp thermal drops as great as 23°, the calves in the non-controlled room showed leucocyte increases as high as 8,000, while the calves in the heat controlled room showed no noticeable changes. To demonstrate that the leucocytes of all calves were capable of responding to sharp thermal drops, the temperature was allowed to drop in both rooms and corresponding high leucocyte counts — as high as 15,000 — for the five individuals were produced by a thermal drop of approximately 20°.

Two specific instances are given indicating that during the prepatent period of stomach worm infection, calves react with considerable difficulty or not at all to decreases in temperature.

200. Observations on starfish, Asterias forbesi, exposed to sea water of reduced salinities. Victor L. Loosanoff, U. S. Fish and Wildlife Service Milford Biological Laboratory.

In three extensive series of experiments large numbers of adult starfish were subjected to dilutions of sea water ranging in salinity from 3 to 20 parts per 1000. In the first series the starfish were placed in various dilutions and left there under observation until their death or the end of the experiment. All the starfish kept in dilution of 16.0 parts per 1000 or less eventually died. The time of death varied from a few hours in very weak dilutions to 2 months in the dilution of 16.0 parts per 1000. In the water, salinity of which was 18.0 and 20.0 parts per 1000, the starfish remained normal for a period of several months.

The second series of experiments was conducted to observe the ability of starfish to recover from short exposures to water of low salinities. Even comparatively brief exposures to the water, salinity of which was less than 16.0 parts per thousand, caused subsequent death to the starfish.

In the third series of experiments an attempt was made to adjust the starfish to low salinities in a very slow and gradual way. Regardless of such gradual changes the starfish failed to adjust themselves to the new conditions. It appears that the salinity of about 18.0 parts per 1000 represents the threshold below which starfish cannot exist. Such a conclusion is in complete agreement with the author's field studies on the distribution of starfish in relation to salinity.

201. Effects of different concentrations of plankton forms upon shell movements, rate of water pumping and feeding and fattening of oysters. Victor L. Loosanoff and James B. Engle, U. S. Fish and Wildlife Service, Milford Biological Laboratory.

Nitzschia closterium, Prorocentrum triangulatum, Euglena viridis, Chlorella sp. and other forms were used in these experiments. In dense concentrations of microorganisms the shell movements of O. virginica become abnormal showing repeated attempts to expel the masses of cells accumulating in the gill cavity. Tonus of the adductor muscle eventually decreases and shells may remain open for days, although no water is pumped by the animals. Responses to stimuli are sluggish.

Light concentrations of microorganisms stimulate the ciliary activities of the gills thus increasing their rate of water pumping. Heavy concentrations, however, interfere with it causing a decrease in the quantity of water pumped. Pumping entirely ceases in very heavy concentrations. Observations indicate that there may be a correlation between the density of the number of microorganisms in sea water and the rate of pumping.

In heavy concentrations no food is ingested by the oysters and the crystalline style is absent. The animals are preoccupied with the formation of large quantities of pseudo-faeces in an attempt to cleanse their gills of the excessive accumulation of microorganisms.

There are definite concentrations of food forms above which the numbers of the microorganisms begin to interfere with the feeding and respiration of the oysters. The animals kept in water too rich in microorganisms fail to fatten and may even die, whereas by subjecting them to the optimum concentration, slightly below the threshold, a very noticeable improvement in their condition can be achieved.

202. A NULL READING PHOTOELECTRIC MICRODENSITOMETER. Joseph G. Baier, Jr., University of Wisconsin.

In all branches of biological research there is an urgent need for better methods of measurements; methods of greater speed; greater sensitivity and greater reliability. The instrument described here was designed to measure the degree of turbidity resulting from mixtures of antibodies and antigens in the precipitin reaction when used in the serological study of animal relationships. Only 1 ml. of solution is necessary and selected 75×10 mm. serological test tubes are used. Data is also presented to illustrate the use of the instrument in the measurement of the density of colored solutions by using the proper color filters.

The instrument employs two RCA photoelectric vacuum tubes in a bridge circuit with amplification. The vacuum tube circuit is stabilized against line voltage fluctuation by means of a built in voltage regulator tube. Readings are obtained by adjusting a wedge, calibrated in per cent light transmission from 100 to 0, to balance the amount of light absorbed by the unknown over the control. The electronic circuit is used only to indicate the balance point. The data is plotted on semi-logarithmic paper. That the data follows the Bouguer-Beer law can be seen from the graphs obtained from a series of dilutions of India ink, barium sulphate and copper sulphate. Once a calibration curve is obtained for any substance, it can always be used for estimating the unknown sample, since the light wedge cannot change its calibration as is possible using direct readings from photocell-meter instruments.

Morphology

203. THE OSTRACOD, CHLAMYDOTHECA UNISPINOSA, REPORTED FROM THE UNITED STATES. Catharine Dobbin Evenson, Smith College. (Introduced by Elizabeth S. Hobbs.)

Specimens of a very large ostracod which the writer would call Chlamydotheca unispinosa (Baird) have been reported for the first time in the United States. In the fall of 1941 two specimens came into the hands of the writer in a culture

of material from the General Biological Supply Co. It was assumed that these had come from the Chicago area. In June 1942 a number of specimens were sent by C. L. Turner from a lagoon of the Chicago River in Cook Co., Illinois. All of these correspond in nearly every detail with the specimens from Yucatan described by Furtos in 1936, with the exception of size. The largest specimen noted so far in the Chicago collection is a female, 4.7 mm. long and 2 mm. high. Others range down to a length of 3 mm., or the more immature ones to a length of 2 mm. No males have as yet come to light. The female of the Yucatan form had a length of 5.6 mm. and a height of 2.5 mm., the male, length 4.8 mm., height 2.3 mm. Since the size of the specimens on hand varies so greatly and since all other features correspond with the Yucatan form, it is assumed that they belong to the same species. The species has been reported also from the Sandwich Islands and Jamaica.

204. COMPARATIVE STUDY OF THE AIR SAC SYSTEM OF BIRDS. Allyn J. Waterman and H. P. Eddy, Williams College.

Preliminary study by the injection method has been made of five domestic birds: pigeon, duck, turkey, guinea hen, chicken. A leg bone is broken and the colored media forced into the open trachea with an all-metal syringe. Lungs and sacs fill readily. Woods metal (Gilbert) gives permanent dry casts easily freed of tissues by maceration but this alloy of low melting point is difficult to handle; mixture of agar and gelatin is not durable; while after hardening vinylite does not fill the cavities and is brittle. Colored latex is satisfactory for dissection, maceration, and permanent display mounting in preserving fluids.

Structurally the systems are grossly similar. Small variations occur in shape, relative size, number of projections, extension, and location of some sacs in different birds. The method demonstrated 3 parts not commonly described: large sacs in the region of the acetabulum; anastomosing passages in synsacrum, keel, and tissues at base of the neck; and in the duck a sac dorsal to the posterior cervical vertebrae. Cervical sac appears paired in pigeon, duck and turkey; single in chicken and guinea hen. Interclavicular varies in size and number of diverticula, and is largest in the duck. Subscapulare may overlap the lung in the pigeon, and generally forms numerous diverticula in the neck. Axillare is most prominent in pigeon. Suprahumerale varies in size especially in pigeon and duck. Sternale is lobulated and largest in pigeon, and consists of anastomosing cavities in hen and duck. Thoracic and abdominal sacs vary widely in size and shape.

205. FURTHER STUDIES OF THE CORTICAL FUSI OF HUMAN-HEAD-HAIR SHAFTS, AND THEIR APPLICATION TO LEGAL WORK. Leon Augustus Hausman, New Jersey College for Women (Rutgers University).

In June, 1942, a paper was presented before Section N of the Association, which reported studies of the microscopic vesicles (fusi) normally occurring in shafts of human head-hair. It was reported that (1) the status of these fusi had proved valuable in establishing personal identification, where only a few shafts (or even fragments) were available, and (2) the condition of the fusi intimated the probable nature of the forces which had brought about these conditions.

In specific court instances of hit-and-run-automobile deaths, fragments of human head-hair shafts, found on fenders, and other parts, (1) correspond in their fusi, with hair-shafts taken from the heads of certain dead individuals, and (2) the occurrence of "artificial" or "fracture" fusi suggested by their numbers, forms, sizes, and dispersal in the cortex of the shafts, the type of maltreatment sustained by the hairs (or fragments) at the time of their deposition on the surfaces where they were found.

Subsequent microscopical studies of hair-shafts, subjected to laboratory experimental maltreatments, and an additional case (December, 1941) of a hit-and-run-automobile death (in which microscopic studies resulted in identification and conviction) further strengthens the view, that natural, and artificial (fracture) fusi of human head-hair shafts are of considerable value, (1) in personal identification, and (2) as appreciable aids in reconstructing certain previous unwitnessed occurrences.

Parasitology

206. Lymphocystis disease in Centrarchidae. J. Richard Weissenberg, Department of Histology, Middlesex University Medical School.

The lymphocystis virus disease of fish is characterized by the gigantic hypertrophy of infected connective tissue cells which are transformed into the so-called lymphocystis cells. In their cytoplasm appear conspicuous reticular inclusion bodies which show a staining reaction like basichromatin. The disease seems to occur rather frequently among the Centrarchidae in the United States. Lymphocystis tumors have been observed in the skin of specimens of Pomoxis nigro-maculatus, Huro salmoides, Micropterus pseudaplites and in several species of Lepomis (gibbosus, macrochirus, megalotis) and in a hybrid of Lepomis cyanellus × macrochirus. Lymphocystis epidemics of L. gibbosus and macrochirus were studied at the Philadelphia Aquarium in fishes taken from Schuylkill River.

As largest size of the uninucleated round lymphocystis cells were 600–670 microns observed in L. gibbosus and macrochirus. As a rule the lymphocystis cells of these two Centrarchidae were shed after the expiration of their growth period so that no macroscopic sign of the healed disease remained. The whole course of the disease took about 16 weeks during the winter in fishes kept in laboratory aquaria without running water.

Usually only one inclusion body appears in the young lymphocystis cells of L. gibbosus and macrochirus. This inclusion passes through a stage in which it resembles a Guarnieri body (characteristic for the Variola virus infection of cells of mammals). Then it sprouts out in the cytoplasm like a mycelium. Later it becomes divided into numerous separate inclusion bodies.

207. Leeches (Ozobranchus branchiatus) on fibro-epithelial tumors of marine turtles (Chelonia mydas). Ross F. Nigrelli, New York Zoological Society.

Large numbers of leeches (Ozobranchus branchiatus) were found attached to papillomata and fibromata removed from the skin of marine turtles (Chelonia mydas). In regions of papilloma where the leeches were more firmly attached,

the keratinized epithelium is thickened and the supporting stroma highly vascularized. The etiology of these tumors is still unknown. Smith and Coates ('39) reported the presence of blood fluke eggs in the stroma and venous or lymphatic spaces of identical tumors. They pointed out that this condition was similar to that found in the papillomatous and malignant tissues in human urinary bilharziasis.

Just what role the ectoparasitic annelids play in the development of these turtle tumors is not certain. They may act as vectors for viral or other parasitic forms that may be the causative agent, although our investigation along these lines has yielded negative results. However, it is altogether possible that the leeches may have some affect on the subsequent growth of the fibro-epithelial tumors. It is known that they feed on the turtle's blood; the latter is prevented from clotting by the action of hirudin. Such a continued flow of blood makes an excellent medium, supplying the necessary nutriment for these slow growing tumor cells. That hirudin, per se, may have some affect on the growth of the tumors is indicated by certain experiments reported in the literature involving the use of heparin in the successful transplantation of goat melanoma, and in tissue culture (see Ligneris, '30, and Zakrzewski, '32).

208. The parasites of the Salamandridae (Amphibia: Caudata). I. A. C. Walton, Knox College, Galesburg, Ill.

Annotated lists of the parasites of the Salamandridae indicate the following hosts and parasites: (a) Euproctus asper (Europe) — Karotomorpha (1) species of Protozoa. (b) Pleurodeles waldklii (Europe) — Chilomastix (1) species of Protozoa. (c) Salamandra atra (Europe) — Aplectana (1), Cosmocerca (1), Oswaldocruzia (1), and Oxysomatium (1) species of Nematoda; Brachycoelium (1) species of Trematoda; Nematotaenia (1) species of Cestoda; Acanthocephalus (1) species of Acanthocephala; Eimeria (2), and Trichomonas (1) species of Protozoa; and Borrelia (1) species of Bacteria (= Spirochaeta of Galli-Valerio, '30). (d) Salamandra palustris (Europe) — Opisthodiscus (1) species of Trematoda. (e) Salamandra salamandra (Europe) — Aplectana (1), Cosmocerca (1), Gordius (1), Oswaldocruzia (1), Oxysomatium (1), and Rhabditis (2) species of Nematoda; Brachycoelium (1), larval Distoma (1), Gorgodera (1), and Opisthioglyphe (1) species of Trematoda; larval Diphyllobothrium (1), Nematotaenia (1), and larval Taenia (1) species of Cestoda; Acanthocephalus (1) species of Acanthocephala; Chilomastix (1), Eimeria (2), Micrococcidium (1), Proteromonas (1), Retortomonas (1), Syndyomita (1), and Trichomonas (2) species of Protozoa; Borrelia (1) species of Bacteria; and Lucilia (1) species of Diptera. (f) Salamanders (Europe) — Saprolegnia (1), and Candida (1) species of Fungi. (g) Salamander larvae (Europe) — Cercaria (1) species of Trematoda. (h) Salamandrina terdigitata (Europe) — Brachycoelium (1) species of Trematoda. (i) Hylototriton andersoni (Japan) — Cosmocercoides (1) species of Nematoda; and Mesocoelium (1) species of Trematoda. Candida batrachea (Scott, '26) is a new combination (= Monilia batrachea Scott, '26).

209. The parasites of the Salamandridae (Amphibia: Caudata). II. A. C. Walton, Knox College, Galesburg, Ill.

Annotated lists of the parasites of the Salamandridae indicate the following hosts and parasites: (a) Triturus alpestris (Europe) — Ascaris (1), Capillaria (1),

Cosmocerca (3), and Hedruris (1) species of Nematoda; Brachycoelium (1), Haematoloechus (1), Opisthioglyphe (1), and Opistodiscus (1) species of Trematoda; Nematotaenia (1) species of Cestoda; Acanthocephalus (1) species of Acanthocephala; Balantidium (1), Eimeria (4), Haptophrya (1), Opalina (1), Tokophrya (1), and Trichomonas (1) species of Protozoa; Dermocystidium (1), and Hepatosphaera (1) species of Fungi. (b) Triturus cristatus (Europe) — Aplectana (1), Ascaris (1), Capillaria (2), Cosmocerca (3), Hedruris (1), Oswaldocruzia (1), and Oxysomoides (1) species of Nematoda; Brachycoelium (1), Digenea larvae (1), Diplodiscus (1), Distoma (1), Opisthioglyphe (1), Opisthodiscus (1), and Pleurogenes (2) species of Trematoda; Acanthocephalus (2) species of Acanthocephala; Balantidium (2), Chloromyxum (1), Dactylosoma (1), Eimeria (5), Hexamita (1), Tokophrya (1), Trichodina (2), and Trichomonas (1) species of Protozoa; Dermocystidium (1), Hepatosphaera (1), and Blastocystis (1) species of Fungi; Lucilia (1) species of Diptera. (c) Triturus helveticus (Europe) — Ascaris (1), and Oswaldocruzia (1) species of Nematoda; Opisthodiscus (1), and Pleurogenes (1) species of Trematoda; Acanthocephalus (2) species of Acanthocephala; Eimeria (3), Entamoeba (2), Hexamita (1), Karotomorpha (1), Protoopalina (1), and Tokophrya (1) species of Protozoa; Dermocystidium (1), Dermomycoides (1), and Hepatosphaera (1) species of Fungi; Lucilia (1) species of Diptera. (d) Triturus marmoratus (Europe) — Brachycoelium (1) species of Trematoda; Balantidium (1), Chilomastix (1), Eimeria (1), and Trichomonas (1) species of Protozoa; Dermocystidium (1) species of Fungi; Lucilia (1) species of Diptera. (e) Triturus meridionalis (U. S. A.) — Cosmocercoides (1) species of Nematoda; Brachycoelium (1), and Manodistomum (1) species of Trematoda.

210. The parasites of the Salamandridae (Amphibia: Caudata). III. A. C. Walton, Knox College, Galesburg, Ill.

Annotated list of the parasites of the Salamandridae indicate the following hosts and parasites: (a) Triturus pyrrhogaster (Japan) — Aplectana (2), Capillaria (2), and Rhabdias (1) species of Nematoda; Brachycoelium (1), Distome cysts (1), Mesocoelium (1), Opisthodiscus (1), and Phyllodistomum (2) species of Trematoda; Acanthocephalus (2) species of Acanthocephala; Balantidium (2), Eimeria (2), Hexamita (1), and Trypanosoma (3) species of Protozoa; Lernaea (2) species of Copepoda. (b) Triturus torosus (U. S. A.) — Cosmocercoides (1), and Hedruris (1) species of Nematoda; Paralechriorchis (1) species of Trematoda; Hexamita (2), Karotomorpha (1), Trichomitus (1), and Trichomonas (2) species of Protozoa. (c) Triturus viridescens (U. S. A.) — Capillaria (4), Cosmocercoides (1), Hedruris (1), Oxyuris (1), Philometra cysts (1), Spinitectus (1), Spiroptera (1), and Spiroxys (1) species of Nematoda; Brachycoelium (1), larval Diplostomulum (1), larval Distoma (1), Gorgoderina (2), Manodistomum (1), Megalodiscus (1), and Plagitura (2) species of Trematoda; Bothriocephalus (1), larval Diphyllobothrium (1), Ophiotaenia (1), and cysts of Proteocephalid (2) species of Cestoda; Acanthocephalus (2), and Pomphorhynchus (1) species of Acanthocephala; Cryptobia (1), Cytamoeba (1), Dactylosoma (1), Entamoeba (1), Euglenomorpha (1), Eutrichomastix (1), Hexamastix (1), Hexamita (3), Karotomorpha (1), Myxobolus (1), Nyctotherus (1), Proteromonas (1), Trichomitus (1), Trichomonas (2), and Trypanosoma (4) species of Protozoa; Achyla (1), and Saprolegnia (1) species of Fungi. (d) Triturus vulgaris

(Europe) — Agamonema (1), Aplectana (1), Ascaris (4), Capillaria (2), Cosmocerca (1), Filaria (2), Hedruris (1), Oswaldocruzia (2), Oxysomatium (1), Oxyuris (1), and Pharyngodon (1) species of Nematoda; Brachycoelium (1), Cercaria (1), larval Distoma (1), Opisthodiscus (1), and Pleurogenes (2) species of Trematoda; larval Diphyllobothrium (1) species of Cestoda; Acanthocephalus (2) species of Acanthocephala; Balantidium (2), Entamoeba (2), Eimeria (2), Hexamastix (1), Leptomonas (1), Mastigamoeba (1), Protoopalina (1), and Tokophrya (1) species of Protozoa; Dermomycoides (1) species of Fungi. (e) Triturus spp? (Europe) — Glossatella (1), Trepomonas (1), and Trichomonas (1) species of Protozoa; Forcipomyia (1) species of Diptera; Euryhelmis (1) species of Trematoda.

Protozoology

211. More mating types and varieties in Paramecium aurelia. T. M. Sonneborn, Indiana University.

In Paramecium aurelia I have previously reported the existence of three non-interbreeding varieties, each containing two interbreeding mating types. Further search has disclosed four more varieties that do not mate with each other or with any of the first three. In each of three of the new varieties two interbreeding mating types occur; in the fourth only one type has thus far been found. Each variety is distinguished not only by its mating types, but also by a unique combination of other characteristics including: conditions for mating, cell size, fission rate, method of inheritance of mating type, interautogamous interval, production of and resistance to lethal substances. For example, variety 4 has the mating types VII and VIII, the smallest cell size, the fastest fission rate (7 to 8 fissions per day at 27°C.), the shortest interautogamous interval (5 to 9 days); it conjugates over a wide range of temperatures and at any hour, shows little change of hereditary mating type at fertilization, and contains races that produce lethal substances. Some of the varieties have been found only once in nature (among collections of paramecia from forty-eight different localities along the east and west coasts and in Indiana). Others have been found repeatedly: variety 1 in fourteen places, variety 2 in eighteen, variety 3 in eight and variety 4 in four places. Probably further search will reveal still more non-interbreeding varieties.

212. Evidence for two distinct mechanisms in the mating reaction of Paramecium aurelia. T. M. Sonneborn, Indiana University.

In variety 1 of Paramecium aurelia one exconjugant of mating type I produced a mutant clone that is still constant after 9 months: it gives typical strong agglutinative mating reactions in mixtures with mating type II, but is unable to go on and fuse in conjugation. The clumps continually form, break up and reform until the animals become too starved to react. Type II individuals that break loose from the clumps sometimes adhere to other individuals of type II in the way typical of preconjugation behavior, but cell fusion and conjugation do not follow. Cell adhesion occurring in the initial stage of the mating reaction and cell fusion occurring during subsequent conjugation are thus due to different mechanisms; for in both of the examples cited above, the former process occurs

while the latter does not. The first process (cell adhesion) appears to be a surface reaction. Further, the substance normally confined to the surface of a type I animal may be transferred to the surface of a type II animal when the two animals adhere to each other and then break apart. This enables the type II animal to behave temporarily like a type I animal, i.e., it may adhere to another type II animal. Other evidence shows also that two type I animals may similarly adhere after one of them has been in contact with a type II animal.

213. Physiology of the ciliate Colpidium colpoda. II. The growth of C. colpoda in nonliving media.[1] William D. Burbanck, Drury College. (Introduced by W. C. Allee.)

On three separate occasions of 1, 5 and 19 weeks sterile colpidia were transferred daily and grown on heat killed Pseudomonas fluorescens and 5% autoclaved brewers' yeast.

Pseudomonas fluorescens was grown on nutrient agar slants for 24 hours; kept for 1 week at 7°C.; then, washed off with 3 ml. of distilled water one of which was added to 100 ml. of distilled water at 60°C. killing the bacterium in 10 minutes. Using 0.5 ml. of dead bacteria and 0.1 ml. of the supernatant centrifuged brewers' yeast, the 0.6 ml. isolation cultures were prepared and in them colpidia were grown at 25.5° ± .5°.

Animals grown on this medium were opaque. The division rate, observed only in groups of from ten to fifty colpidia, never exceeded more than one division in 48 hours. Prepared yeast and bacteria showed no apparent aging effect in 10 days. Pseudomonas killed at 60°C. in an hour plus yeast supported only one division in 2 weeks. Unsuccessful attempts were made to grow mass cultures in buffered and unbuffered media. Experimental colpidia grown on living Pseudomonas again divided at a normal rate.

Two colpidia grown in plasmoptysate of a marine P. fluorescens[2] and brewers' yeast produced about 200 animals in 2 weeks.

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[1] I wish to thank the Zoology Department of Columbia University for the use of their facilities.

[2] Obtained from Prof. C. V. Taylor of Stanford University.

214. Growth of populations in relation to size of the inoculum in cultures of Glaucoma piriformis. R. P. Hall and W. B. Cosgrove, New York University.

Populations started with various initial densities have been compared during the first 7 days of incubation in several culture media. In general, an initial period of rapid multiplication, which may or may not be preceded by an initial stationary phase, has been followed by a marked decline in division-rate, and the lower division-rates have persisted throughout the period of observation. Populations with small initial densities and those with initial densities more than 100 times as great have shown comparable behavior. Low-initial populations have entered a "phase of negative growth acceleration" at densities ranging from approximately 300 to 1000 ciliates per cubic centimeter, while high-initial populations enter such a phase at a much greater density. The results suggest that attainment of a particular density of population is not the factor primarily responsible for the marked decline in division-rate which follows the early phase of rapid multiplication.

215. Mineral deficiency as a factor limiting growth of the ciliate, Glaucoma piriformis. R. P. Hall and W. B. Cosgrove, New York University.

"Vitamin-free" casein (Casein-Harris) in 1% solution failed to support growth of G. piriformis for more than one transfer. Neither thiamin nor a combination of thiamin and riboflavin served as an adequate supplement to the basal medium, and the ciliates failed to grow after the first transfer just as in the "vitamin-free" control medium. However, following the addition of several salts (including K_2HPO_4, $MgSO_4$ and $CaCl_2$) to a 1% solution of Casein-Harris, growth of the ciliates has now been maintained for nine serial transfers with no indications of dietary deficiency. Obviously, the limiting factor in this "vitamin-free" casein medium, so far as G. piriformis is concerned, is a mineral deficiency rather than a vitamin deficiency. Since Dewey ('41) has used Casein-Harris in 1% solution as a basal medium, our findings may have some bearing on her conclusion that Tetrahymena geleii probably requires two new growth-factors.

216. The comparative in vitro effect of various sulfonamide drugs on Leishmania tropica. Alfred M. Elliott, Minnesota State Teachers College, Bemidji.

The flagellates were cultured in tubes containing 5 cc. of N.N.N. medium with citrated rabbit blood. To this was added a 2% peptone solution (pH 6.8) containing the sulfonamides in 10 mg. and 100 mg. per cent concentrations. The sulfa drugs employed were: sulfanilamide, sulfaguanidine, sulfathiazole, and the sodium salts of sulfadiazine and sulfapyridine. Comparative studies were made with antimony potassium tartrate (tartar emetic).

After several series of experiments it was found that none of the drugs were effective in retarding the growth of this flagellate. In tubes containing 10 mg. per cent of the drugs slight stimulation of growth was observed; in tubes containing 100 mg. per cent there was no appreciable effect of any of the drugs. Tartar emetic inhibited all growth at both concentrations. From these observations the five commonly used sulfonamide drugs are apparently ineffective in inhibiting the growth of Leishmania tropica in vitro.

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